GNE.3230R1C39 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Eaton, et al.
Appl. No.	:	10/063,557
Filed	:	May 2, 2002
For	:	SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME
Examiner	:	David J. Blanchard
Group Art Unit	:	1642

DECLARATION OF J. CHRISTOPHER GRIMALDI, UNDER 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, J. Christopher Grimaldi, declare and state as follows:

1. I am a Senior Research Associate in the Molecular Biology Department of Genentech, Inc., South San Francisco, CA 94080.

2. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

3. I joined Genentech in January of 1999. From 1999 to 2003, I directed the Cloning Laboratory in the Molecular Biology Department. During this time I directed or performed numerous molecular biology techniques including semi-quantitative Polymerase Chain Reaction (PCR) analyses. I am currently involved, among other projects, in the isolation of genes coding for membrane associated proteins which can be used as targets for antibody therapeutics against cancer. In connection with the above-identified patent application, I personally performed or directed the semi-quantitative PCR gene expression analyses in the assay entitled "Tumor Versus Normal Differential Tissue Expression Distribution," which is described in EXAMPLE 18 in the specification. These studies were used to identify differences in gene expression between tumor tissue and their normal counterparts.

4. EXAMPLE 18 reports the results of the PCR analyses conducted as part of the investigating of several newly discovered DNA sequences. This process included developing

primers and analyzing expression of the DNA sequences of interest in normal and tumor tissues. The analyses were designed to determine whether a difference exists between gene expression in normal tissues as compared to tumor in the same tissue type.

5. The DNA libraries used in the gene expression studies were made from pooled samples of normal and of tumor tissues. Data from pooled samples is more likely to be accurate than data obtained from a sample from a single individual. That is, the detection of variations in gene expression is likely to represent a more generally relevant condition when pooled samples from normal tissues are compared with pooled samples from tumors in the same tissue type.

6. In differential gene expression studies, one looks for genes whose expression levels differ significantly under different conditions, for example, in normal versus diseased tissue. Thus, I conducted a semi-quantitative analysis of the expression of the DNA sequences of interest in normal versus tumor tissues. Expression levels were graded according to a scale of +, -, and +/- to indicate the amount of the specific signal detected. Using the widely accepted technique of PCR, it was determined whether the polynucleotides tested were more highly expressed, less expressed, or whether expression remained the same in tumor tissue as compared to its normal counterpart. Because this technique relies on the visual detection of ethidium bromide staining of PCR products on agarose gels, it is reasonable to assume that any detectable differences seen between two samples will represent at least a two fold difference in cDNA.

7. The results of the gene expression studies indicate that the genes of interest can be used to differentiate tumor from normal. The precise levels of gene expression are irrelevant; what matters is that there is a relative difference in expression between normal tissue and tumor tissue. The precise type of tumor is also irrelevant; again, the assay was designed to indicate whether a difference exists between normal tissue and tumor tissue of the same type. If a difference is detected, this indicates that the gene and its corresponding polypeptide and antibodies against the polypeptide are useful for diagnostic purposes, to screen samples to differentiate between normal and tumor. Additional studies can then be conducted if further information is desired.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: _____ Date: 8/10/2004

J. Christopher Grimaldi

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1434-36th Ave.
San Francisco, CA 94122
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EDUCATION University of California, Berkeley
Bachelor of Arts in Molecular Biology, 1984

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3. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

4. In differential gene expression studies, one looks for genes whose expression levels differ significantly under different conditions, for example, in normal versus diseased tissue.

Chromosomal aberrations, such as gene amplification, and chromosomal translocations are important markers of specific types of cancer and lead to the aberrant expression of specific genes and their encoded polypeptides, including over-expression and under-expression. For example, gene amplification is a process in which specific regions of a chromosome are duplicated, thus creating multiple copies of certain genes that normally exist as a single copy. Gene under-expression can occur when a gene is not transcribed into mRNA. In addition, chromosomal translocations occur when two different chromosomes break and are rejoined to each other chromosome resulting in a chimeric chromosome which displays a different expression pattern relative to the parent chromosomes. Amplification of certain genes such as Her2/Neu [Singleton *et al.*, Pathol. Annu., 27Pt1:165-190], or chromosomal translocations such as t(5;14), [Grimaldi *et al.*, Blood, 73(8):2081-2085(1989); Meeker *et al.*, Blood, 76(2):285-289(1990)] give cancer cells a growth or survival advantage relative to normal cells, and might also provide a mechanism of tumor cell resistance to chemotherapy or radiotherapy. When the chromosomal aberration results in the aberrant expression of a mRNA and the corresponding gene product (the polypeptide), as it does in the aforementioned cases, the gene product is a promising target for cancer therapy, for example, by the therapeutic antibody approach.

5. Comparison of gene expression levels in normal versus diseased tissue has important implications both diagnostically and therapeutically. For example, those who work in this field are well aware that in the vast majority of cases, when a gene is over-expressed, as evidenced by an increased production of mRNA, the gene product or polypeptide will also be over-expressed. It is unlikely that one identifies increased mRNA expression without associated increased protein expression. This same principle applies to gene under-expression. When a gene is under-expressed, the gene product is also likely to be under-expressed. Stated in another way, two cell samples which have differing mRNA concentrations for a specific gene are expected to have correspondingly different concentration of protein for that gene. Techniques used to detect mRNA, such as Northern Blotting, Differential Display, *in situ* hybridization, quantitative PCR, Taqman, and more recently Microarray technology all rely on the dogma that a change in mRNA will represent a similar change in protein. If this dogma did not hold true then these techniques would have little value and not be so widely used. The use of mRNA quantitation techniques have identified a seemingly endless number of genes which are differentially expressed in various tissues and these genes have subsequently been shown to have correspondingly similar changes in their protein levels. Thus, the detection of increased mRNA expression is expected to result in increased polypeptide expression, and the detection of decreased mRNA expression is expected to result in decreased polypeptide expression. The detection of increased or decreased polypeptide expression can be used for cancer diagnosis and treatment.

6. However, even in the rare case where the protein expression does not correlate with the mRNA expression, this still provides significant information useful for cancer diagnosis and treatment. For example, if over- or under-expression of a gene product does not correlate with over- or under-expression of mRNA in certain tumor types but does so in others, then identification of both gene expression and protein expression enables more accurate tumor classification and hence better determination of suitable therapy. In addition, absence of over- or

under-expression of the gene product in the presence of a particular over- or under-expression of mRNA is crucial information for the practicing clinician. For example, if a gene is over-expressed but the corresponding gene product is not significantly over-expressed, the clinician accordingly will decide not to treat a patient with agents that target that gene product.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

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J. Christopher Grimaldi

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The t(5;14) Chromosomal Translocation in a Case of Acute Lymphocytic Leukemia Joins the Interleukin-3 Gene to the Immunoglobulin Heavy Chain Gene

By J. Christopher Grimaldi and Timothy C. Meeker

Chromosomal translocations have proven to be important markers of the genetic abnormalities central to the pathogenesis of cancer. By cloning chromosomal breakpoints one can identify activated proto-oncogenes. We have studied a case of B-lineage acute lymphocytic leukemia (ALL) that was associated with peripheral blood eosinophilia. The chromosomal translocation t(5;14) (q31;q32) from this sample was cloned and studied at the molecular level. This translocation joined the immunoglobulin heavy chain joining (Jh) region to the promotor region of the interleukin-3 (IL-3) gene in opposite transcriptional orientations. The data suggest that activation of the IL-3 gene by the enhancer of the immunoglobulin heavy chain gene may play a central role in the pathogenesis of this leukemia and the associated eosinophilia.

KARYOTYPIC STUDIES of leukemia and lymphoma have identified frequent nonrandom chromosomal translocations. Some of these translocations juxtapose the immunoglobulin heavy chain (IgH) gene with important protooncogenes, such as c-*myc* and *bcl*-2.[1,2] In this way, the IgH gene can activate proto-oncogenes, resulting in disordered gene expression and a step in the development of cancer. The investigation of additional nonrandom translocations into the IgH locus allows us to identify new genes promoting the generation of leukemia and lymphoma.

A distinct subtype of acute lymphocytic leukemia (ALL) has been characterized by B-lineage phenotype, associated eosinophilia in the peripheral blood, and a t(5;14)(q31;q32) chromosomal translocation.[3,4] This syndrome probably occurs in <1% of all patients with ALL. We hypothesized that the cloning of the translocation characteristic of this leukemia might allow the identification of an important gene on chromosome 5 that plays a role in the evolution of this disease. In this report we demonstrate that the interleukin-3 gene (IL-3) and the IgH gene are joined by this translocation.

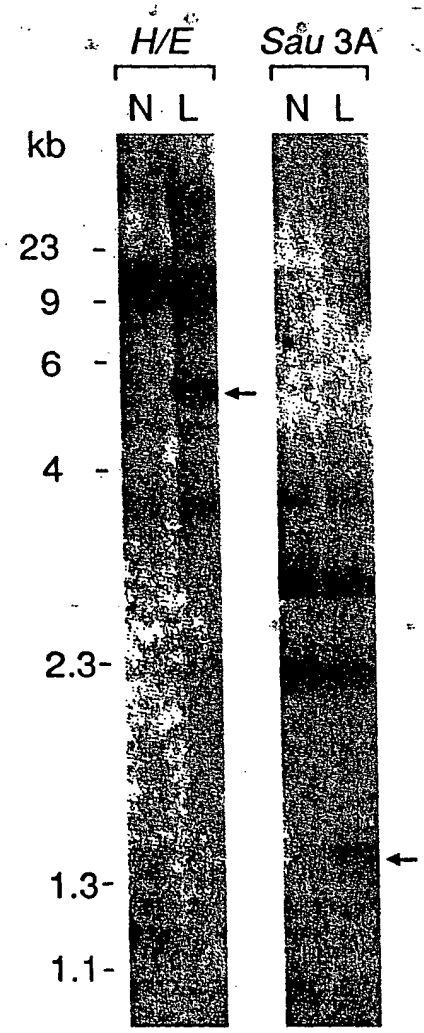

Fig 1. DNA blots of the leukemia sample. The restriction fragment pattern of normal human DNA (N) and the leukemia sample (L) were compared using a human Jh probe. Rearranged bands are indicated by arrows. Sample L exhibits a single rearranged band with both *Hind* III/*Eco*RI and *Sau*3A restriction digests. The rearranged bands are less intense than the other bands because the majority of cells in the sample represent normal bone marrow elements.

MATERIALS AND METHODS

Sample and DNA blots. A bone marrow aspirate from a representative patient with ALL (L1 morphology by French-American-British [FAB] criteria), peripheral eosinophilia (up to 20,000 per microliter with a normal value of <350 per microliter) and a t(5;14)(q31;q32) translocation was studied. Using published methods, genomic DNA was isolated and DNA blots were made.[5] Briefly, 10 μg of high molecular weight (mol wt) DNA were digested using an appropriate restriction enzyme and electrophoresed on a 0.8% agarose gel. The gel was stained with ethidium bromide, photographed, denatured, neutralized, and transferred to Hybond (Amersham, Arlington Heights, IL). After treatment of the filter with ultraviolet light, hybridization was performed. The filter was washed to a final stringency of 0.2% saturated sodium citrate (SSC) and 0.1% sodium lauryl sulfate (SDS) and exposed to film. The human Jh probe has been previously reported.[6]

Genomic library. The genomic library was made using pub-

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lished methods.[5] Approximately 100 μg of high mol wt genomic DNA were partially digested with the *Sau*3A restriction enzyme. Fragments from 9 to 23 kilobases (kb) in size were isolated on a sucrose gradient and ligated into phage EMBL3A (Strategene, San Diego). Recombinant phage were packaged, plated, and screened as previously reported.[5]

DNA sequencing. Fragments for sequencing were cloned into M13 vectors and sequenced by the chain termination method using Sequenase (United States Biochemical, Cleveland).[7] All sequence data were derived from both strands.

RESULTS

We studied a bone marrow sample from a patient with ALL and associated peripheral eosinophilia. Karyotypic analysis showed the characteristic t(5;14)(q31;q32) translocation. These features define a distinctive subtype of ALL.[3,4] The leukemic cells were analyzed for cell surface phenotype by immunofluorecence. They were positive for B1 (CD20), B4 (CD19), cALLA (CD10), HLA-DR, and terminal deoxynucleotidyl transferase (Tdt), but negative for surface immunoglobulin. This phenotypic profile describes an immature cell from the B-lymphocytic lineage.[8]

The leukemia DNA was analyzed by Southern blotting for rearrangements of the IgH gene. Using a human immunoglobulin Jh probe, a single rearranged band was detected by *Eco*RI, *Hind*III, *Sst*I, *Sau*3A, and *Eco*RI plus *Hind*III restriction digests, suggesting rearrangement of one allele (Fig 1). The immunoglobulin Jh region from the other allele was presumably either deleted or in the germline configuration.

We hypothesized that the t(5;14)(q31;q32) juxtaposed a growth-promoting gene on chromosome 5 with the immunoglobulin Jh region on chromosome 14. Therefore, a genomic library was made from the leukemic sample and screened with a Jh probe. Fifteen distinct positive clones were isolated and screened for the presence of the rearranged *Sau*3A fragment that was detected by DNA blotting. By this analysis, five clones appeared to represent the rearranged allele identified by DNA blots. One of these clones (clone no. 4) was chosen for further study and a detailed restriction map was generated. The *Eco*RI, *Hind*III/*Eco*RI, and *Sst*I fragments from clone no. 4 that hybridized to the human Jh probe were also identical in size to the rearranged fragments from the leukemia sample, confirming that clone no. 4 represented the rearranged leukemic allele.

Phage clone no. 4 contained 3.7 kb of unknown origin joined to the IgH gene in the region of Jh4 (Fig 2). The IgH gene from Jh4 to the Cmu region appeared to be in germline configuration. Previously, the gene encoding hematopoietic growth factor IL-3 had been mapped to chromosome 5q31 so it was suspected that clone no. 4 might contain part of this gene.[9-12] When the restriction map of human IL-3 and clone no. 4 were compared, they were identical for more than 3 kb (Fig 2).

We confirmed the juxtaposition of the IL-3 gene and the IgH gene by nucleic acid sequencing of the subcloned *Bst*EII/*Hpa*I fragment (Fig 2). The sequence of this fragment showed no disruption of the protein coding region or the messenger RNA of the IL-3 gene. The break in the IL-3 gene occurred in the promotor region, 452 base pairs (bp) upstream of the transcriptional start site (position 64, Fig

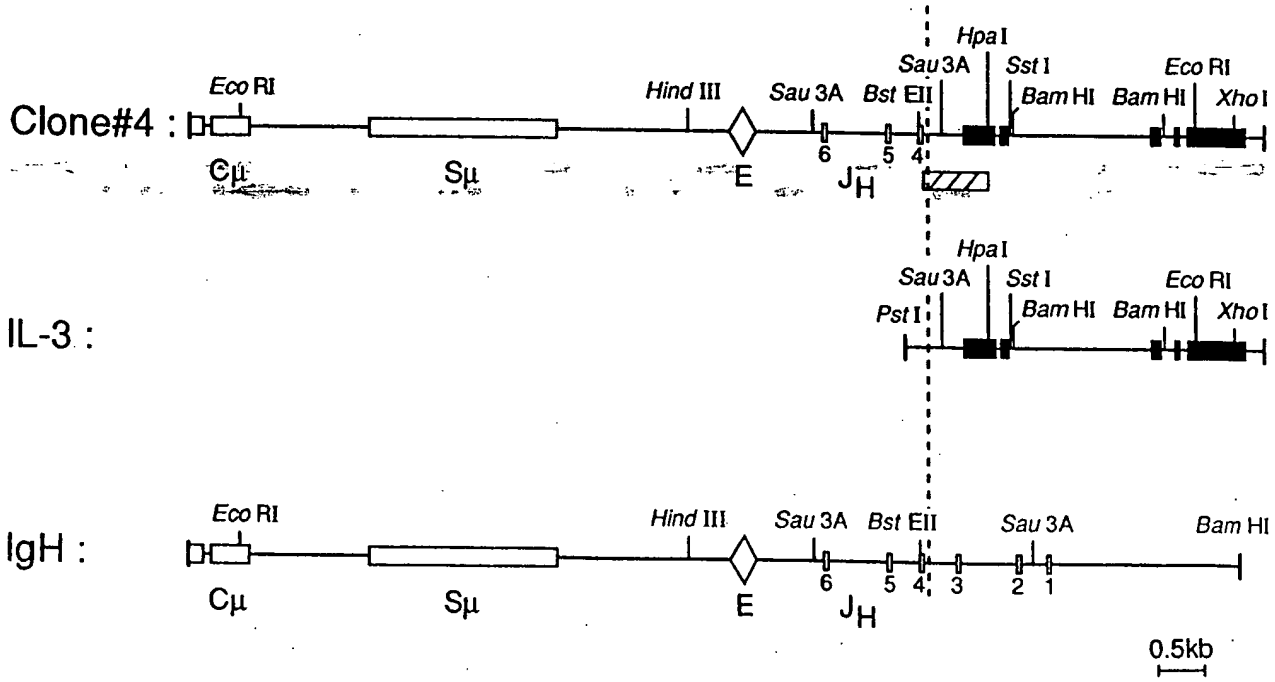

Fig 2. Breakpoint region: t(5;14)(q31;q32). Comparative mapping of phage clone no. 4, the germline IgJh region, and the germline IL-3 gene.[28,29] The map of clone no. 4 is identical to that of IgH until it diverges in the region of Jh4 (at the dashed line), after which it is identical to the map of IL-3. The two genes are positioned in a head-to-head orientation. The Ig μ chain constant region (Cμ), switch region (Sμ), enhancer (E), and Jh segments are indicated (open symbols). The five exons (dark boxes) and four introns of the IL-3 gene are shown. The hatched box indicates the sequenced region.

3A). The break in the IgH gene occurred 2 bp upstream of the Jh4 region. Between the two breaks, 25 bp of uncertain origin (putative N sequence) were inserted.[13,14] No sequences homologous to the immunoglobulin heptamer and nonamer could be identified in the IL-3 sequence (Fig 3B). Therefore, nucleic acid sequencing confirmed the juxtaposition of the IL-3 gene and the IgH gene. The sequence data clearly showed that the genes were positioned in opposite transcriptional orientations (head-to-head).

Available data also allowed us to determine the normal positions of the IL-3 gene and the GM-CSF gene in relation to the centromere of chromosome 5 (Fig 4). The IgH gene is known to be positioned with the variable regions toward the telomere on chromosome 14q.[2,15] It has also been shown that GM-CSF maps within 9 kb of IL-3 in the same transcriptional orientation.[16] Using this information and assuming a simple translocation event in our sample, we can conclude that the IL-3 gene is normally more centromeric, and the GM-CSF gene more telomeric on chromosome 5q (Fig 4). Furthermore, both are transcribed with their 5′ ends toward the centromere.

DISCUSSION

In this report we have cloned a unique chromosomal translocation that appears to be a consistent feature of a rare, yet distinct, clinical form of acute leukemia. This translocation joined the promotor of the IL-3 gene to the IgH gene. Except for the altered promotor, the IL-3 gene appeared

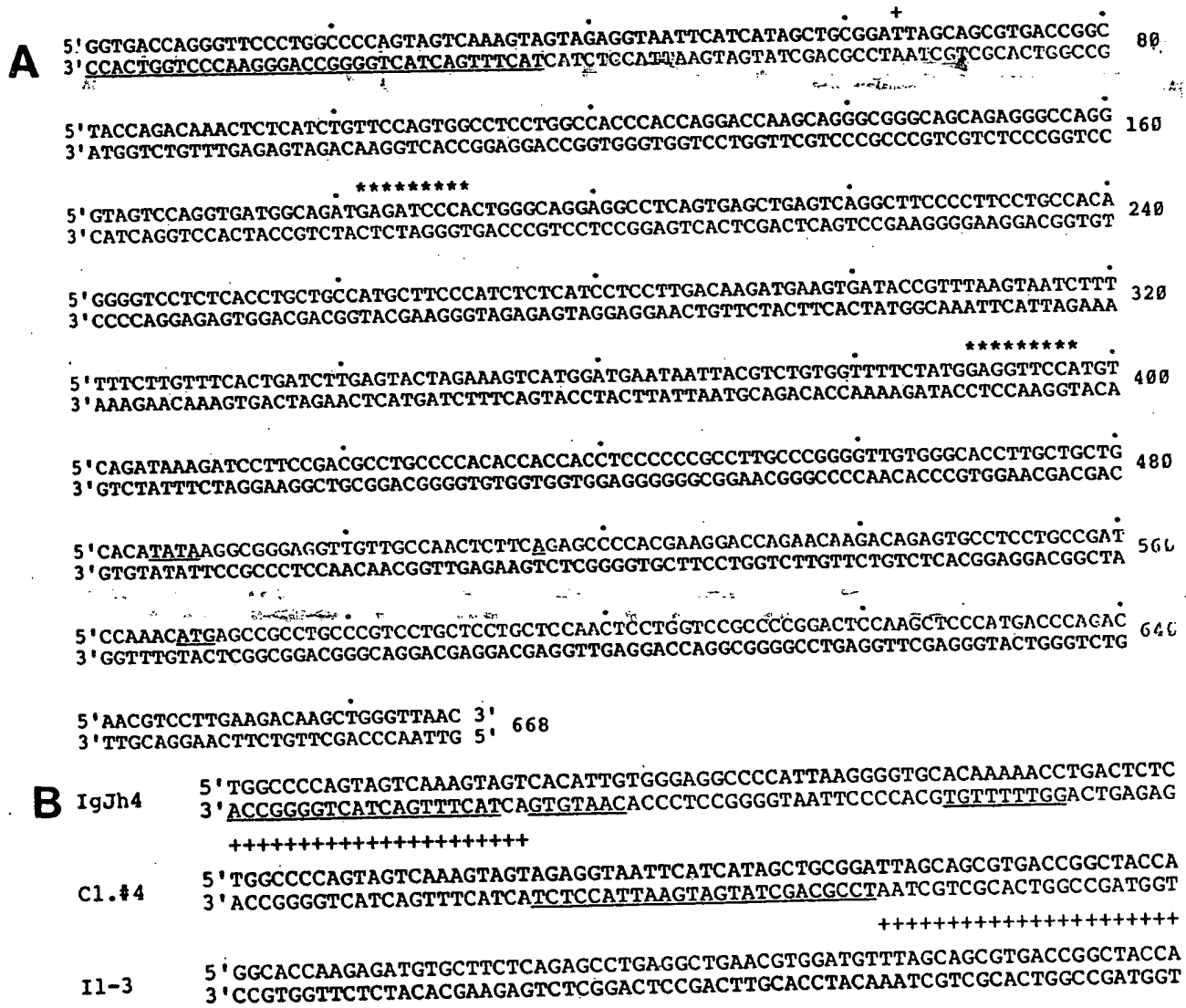

Fig 3. Sequence of t(5;14)(q31;q32) breakpoint region. (A) Nucleotide sequence of the *Bst*EII/*Hpa*I fragment indicated on Fig 2. Nucleotides 1 to 36 represent the Jh4 coding region underlined on the coding strand.[6] Nucleotides 39 to 63 are a putative N region. The sequence from position 64 to 668 is that of the germline IL-3 gene.[28] The IL-3 TATA box (485), transcription start (515), and initiation methionine (567) are underlined. Two proposed regulatory sequences in the promotor are marked by asterisks (positions 182 and 389). (B) Comparative sequence of the t(5;14)(q31;q32) breakpoint region. The IgJh4 region is shown with its coding region, heptamer, and nonamer underlined. Clone no. 4 is shown with putative N region sequences underlined. The IL-3 sequence is also shown. A plus sign (+) denotes the identical nucleotide between sequences. No heptamer or nonamer is identified in the IL-3 sequence.

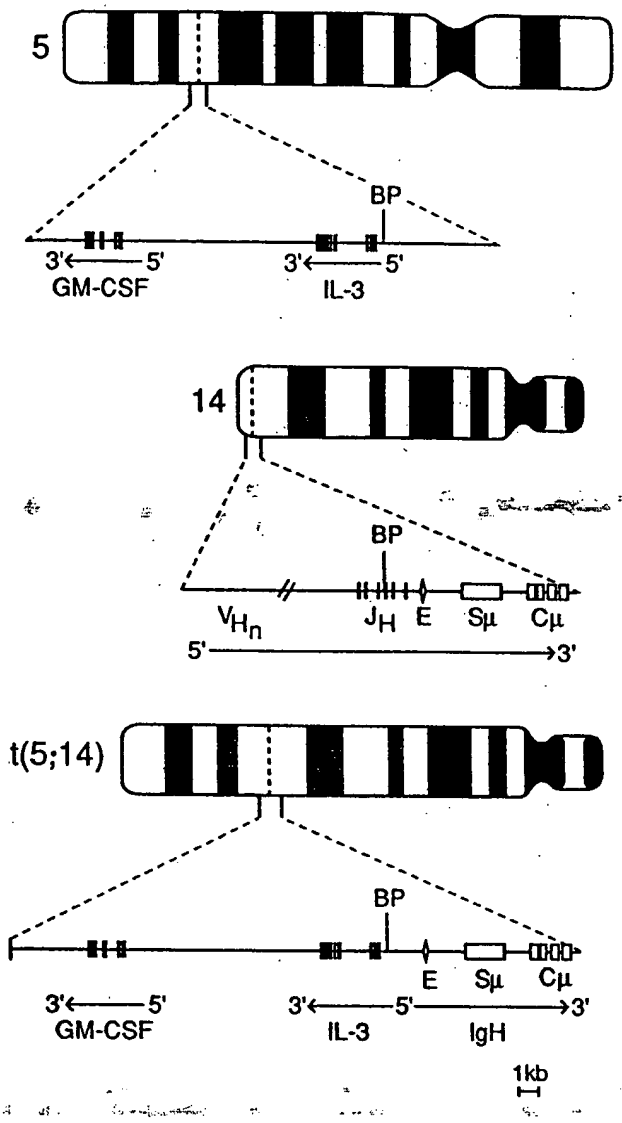

Fig 4. Diagram of the translocation. The normal chromosome 5q31 is shown with the GM-CSF gene telomeric to the IL-3 gene in the transcriptional orientation shown. On normal chromosome 14q32 the Vh regions are telomeric. The t(5;14)(q31;q32) translocation results in the head-to-head orientation of these genes. Symbols are defined in Fig 2. BP, breakpoint position.

intact as no deletions, insertions, or point mutations were detected by restriction mapping of the entire gene and sequencing of part of the gene. The IgH gene has been truncated at the Jh4 region, which places the immunoglobulin enhancer within 2.5 kb of the IL-3 gene.[17,18] This leads to the hypothesis that the enhancer is increasing transcription of a structurally normal IL-3 gene. The same mechanism is important for activation of the c-*myc* gene in some cases of Burkitt's lymphoma.[19] An alternate hypothesis is that the elimination of an upstream IL-3 promotor element is crucial to the activation of the IL-3 gene.

The proposed activation of the IL-3 gene suggests that an autocrine loop is important for the pathogenesis of this leukemia.[20] Over-expression of the IL-3 gene coupled with the presence of the IL-3 receptor in these cells could account for a strong stimulus for proliferation. In this regard, there are data indicating that immature B-lineage lymphocytes and B-lineage leukemias may express the IL-3 receptor.[21,22]

An additional feature of this type of leukemia is the dramatic eosinophilia, consisting of mature forms. It has been hypothesized that the eosinophils do not arise from the malignant clone, but are stimulated by the tumor.[23,24] Because of the known effect of IL-3 on eosinophil differentiation, secretion of high levels of IL-3 by leukemic cells might have a role in the eosinophilia in this type of leukemia.[12]

The data suggest that the recombination mechanism that is active in the IgH gene during normal differentiation has a role in this translocation.[13,14] This is supported by the breakpoint location at the 5′ end of Jh4 and the presence of putative N-region sequences. On the other hand, no recombination signal sequence (heptamer and nonamer) was found in this region on chromosome 5, suggesting that additional factors also played a role. Further studies will elucidate the mechanism of this and other translocations.

In the leukemia we studied, it is possible that the immunoglobulin enhancer also activates the GM-CSF gene, since this gene is probably positioned only 14 kb away (Fig 4). This is known to be within the range of enhancer activation.[25] The interleukin-5 (IL-5) gene maps to chromosome 5q31.[26] Deregulation of the IL-5 gene by this translocation would act synergistically with IL-3 in the stimulation of eosinophil proliferation and differentiation.[27] These and other questions will be answered by the study of more patient samples. We plan to determine whether the t(5;14)(q31;q32) translocation is capable of activating multiple lymphokines simultaneously and whether they cooperate in the generation of this leukemia.

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RAPID COMMUNICATION

Activation of the Interleukin-3 Gene by Chromosome Translocation in Acute Lymphocytic Leukemia With Eosinophilia

By Timothy C. Meeker, Dan Hardy, Cheryl Willman, Thomas Hogan, and John Abrams

The t(5;14)(q31;q32) translocation from B-lineage acute lymphocytic leukemia with eosinophilia has been cloned from two leukemia samples. In both cases, this translocation joined the IgH gene and the interleukin-3 (IL-3) gene. In one patient, excess IL-3 mRNA was produced by the leukemic cells. In the second patient, serum IL-3 levels were measured and shown to correlate with disease activity. There was no evidence of excess granulocyte/macrophage colony stimulating factor (GM-CSF) or IL-5 expression. Our data support the formulation that this subtype of leukemia may arise in part because of a chromosome translocation that activates the IL-3 gene, resulting in autocrine and paracrine growth effects.

A NUMBER OF chromosome translocations have been associated with human leukemia and lymphoma. In many cases the study of these translocations has led to the discovery or characterization of proto-oncogenes, such as *bcl*-2, c-*abl*, and c-*myc*, that are located adjacent to the translocation.[1,2] It is now widely understood that cancer-associated translocations disrupt nearby proto-oncogenes.

A distinct subtype of acute leukemia is characterized by the triad of B-lineage immunophenotype, eosinophilia, and the t(5;14)(q31;q32) translocation.[3,4] Leukemic cells from such patients have been positive for terminal deoxynucleotidyl transferase (Tdt), common acute lymphoblastic leukemia antigen (CALLA), and CD19, but negative for surface or cytoplasmic immunoglobulin. In previous work, we cloned the t(5;14) breakpoint from one leukemic sample (Case 1) and determined that the IgH and interleukin-3 (IL-3) genes were joined by this abnormality.[5] In this report, we extend those findings by showing that the t(5;14)(q31;q32) translocation from a second leukemia sample (Case 2) has a similar structure, and we report our study of growth factor expression in these patients.

MATERIALS AND METHODS

Samples and Southern blots. Case 1 has been described.[5,6] Clinical features of Case 2 have been described in detail.[3] DNA isolation and Southern blotting was done using previously described methods.[5] Filters were hybridized with an immunoglobulin Jh probe, a 280 bp *Bam*HI/*Eco*RI genomic IL-3 fragment, and an IL-3 cDNA probe.[7,8]

Northern blots. RNA isolation and Northern blotting have been described.[9] Briefly, Northern blots were done by separating 9 μg total RNA on 1% agarose-formaldehyde gels. Equal RNA loading in each lane was confirmed by ethidium bromide staining. Blots were hybridized with an IL-3 cDNA probe extending to the *Xho* I site in exon 5, a 720 bp *Sst* I/*Kpn* I probe derived from intron 2 of the IL-3 gene, a 600 bp *Nhe* I/*Hpa* I IL-5 cDNA probe, and a 500 bp *Pst* I/*Nco* I granulocyte-macrophage colony stimulating factor (GM-CSF) cDNA probe.[10-12]

Polymerase chain reaction. Primers were designed with *Bam*HI sites for cloning. One primer hybridized to the Jh sequences from the IgH gene (Primer 144:5'-TAGGATCCGACGGTGACCAGGGT), and the other hybridized to the region of the TATA box in the IL-3 gene (Primer 161: 5'-AACAGGATCCCGCCTTATATGTGCAG). Polymerase chain reaction (PCR) (95°C for 1 minute, 61°C for 30 seconds, and 72°C for 3 minutes) was done using 500 ng genomic DNA and 50 pmol of each primer in 100 μL containing 67 mmol/L Tris-HCl pH 8.8, 6.7 mmol/L $MgCl_2$, 10% dimethyl sulfoxide (DMSO), 170 μg/mL bovine serum albumin (BSA) (fraction V), 16.6 mmol/L ammonium sulfate, 1.5 mmol/L each dNTP and Taq polymerase (Perkin-Elmer, Norwalk, CT).[13]

Sequencing. Sequencing was done by chain termination in M13 vectors.[14] As part of this study, we sequenced a subclone of a normal IL-3 promotor, covering 598 base pairs from a *Sma* I site at position −1240 (with respect to the proposed site of transcription initiation) to an *Nhe* I site at position −642. The plasmid containing this region was a gift from Naoko Arai of the DNAX Research Institute.

Expression in Cos7 cells. A genomic IL-3 fragment from Case 1 was cloned into the pXM expression vector.[10] Briefly, the *Hin*dIII/*Sal* I fragment containing the IL-3 gene was subcloned from the previously described phage clone 4 into pUC18.[5] The 2.6 kb fragment extending from the *Sma* I site 61 bp upstream of the IL-3 transcription start to the *Sma* I site in the polylinker was cloned into the blunted *Xho* I site of pXM. The negative control construct was the pXM vector without insert. Plasmids were introduced into Cos7 cells by electroporation, and supernatant was collected after 48 hours in culture.

TF1 bioassay. TF-1 cells were passaged in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol L-glutamine, and 1 ng/mL human GM-CSF.[15] Samples and antibodies were diluted in this same medium lacking GM-CSF but containing penicillin and streptomycin. A 25 μL volume of serial dilutions of patient serum was added to wells in a flat bottom 96-well microtiter plate. Rat anti-cytokine monoclonal antibody in a volume of 25 μL was added to appropriate wells and preincubated for 1 hour at 37°C. Fifty microliters of twice washed TF-1 cells were added to each well, giving a final cell concentration of 1×10^4 cells per well (final volume, 100 μL). The plate was incubated for 48 hours. The remaining cell viability was determined metabolically by the colori-

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IgJh5   5'GACGGTGACCAGGGTTCCTTGGCCCCAGGAGTCGAACCAGTTGTCACATTGTGACAACAATGCCAGACCCCGACAAAGAACGGGCGCCCCGCACGTCCCT
        3'CTGCCACTGGTCCCAAGGAACCGGGGTCCTCAGCTTGGTCAACAGTGTAACACTGTTGTTACGGTCTGGGGCTGTTTCTTGCCCGCGGGGCGTGCAGGGA
          ++++++++++++++++++ ++++++++++ +++++++++++++++

Clone   5'GACGGTGACCAGGGTTCCCTGGCCCCAGGGGTCGAACCAGTTGTACGGCCATGTTATTGGGGGGGATCAAGACCTCTCAATACAACTGTCTCCTGCCAAT
        3'CTGCCACTGGTCCCAAGGGACCGGGGTCCCCAGCTTGGTCAACATGGCGGTACAATAACCCCCCCTAGTTCTGGAGAGTTATGTTGACAGAGGACGGTTA
                                                                    (-934)*++++++++++++++++++++++++++++++++++++++++

IL3     5'CCCCTCTCTGCAAACCTTGCCTACTGGGCCTGCACCTGGCAAATCCATGCTCAGCACAGACGGGGATCAAGACCTCTCAATACAACTGTCTCCTGCCAAT
        3'GGGGAGAGACGTTTGGAACGGATGACCCGGACGTGGACCGTTTAGGTACGAGTCGTGTCTGCCCCTAGTTCTGGAGAGTTATGTTGACAGAGGACGGTTA
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Fig 1. Breakpoint sequences for Case 2. The germline IgJh5 region sequence (protein coding region and recombination signal sequences are underlined) is on top, the translocation sequence from Case 2 (PCR primer sequences and putative N region are underlined) is in the middle, and the germline IL-3 sequence, which we derived from a normal IL-3 clone, is on the bottom.[7] + indicates that each sequence has the same nucleotide. The sequence documents the head-to-head joining of the IL-3 and IgH genes. The breakpoint in the IL-3 gene occurred at position −934 (*).

metric method of Mosmann using a VMax microtiter plate reader (Molecular Devices, Menlo Park, CA) set at 570 and 650 nm.[16]

Cytokine immunoassays. These assays used rat monoclonal anti-cytokine antibodies (10 μg/mL) to coat the wells of a PVC microtiter plate. The capture antibodies used were BVD3-6G8, JES1-39D10, and BVD2-23B6, for the IL-3, IL-5, and GM-CSF assays, respectively. Patient sera were then added (undiluted and diluted 1:2 for IL-3, undiluted for IL-5, and undiluted and diluted 1:5 for GM-CSF). The detecting immunoreagents used were either mouse antiserum to IL-3 or nitroiodophenyl (NIP)-derivatized rat monoclonal antibodies JES1-5A2 and BVD2-21C11, specific for IL-5 and GM-CSF, respectively. Bound antibody was subsequently detected with immunoperoxidase conjugates: horseradish peroxidase (HRP)-labeled goat anti-mouse Ig for IL-3, or HRP-labeled rat (J4 MoAb) anti-NIP for IL-5 and GM-CSF. The chromogenic substrate was 3-3'azino-bis-benzthiazoline sulfonate (ABTS; Sigma, St Louis, MO). Unknown values were interpolated from standard curves prepared from dilutions of the recombinant factors using Softmax software available with the VMAX microplate reader (Molecular Devices).

RESULTS

Leukemic DNA from Case 2 was studied by Southern blotting. When digested with the *Hin*dIII restriction enzyme and hybridized with a human immunoglobulin heavy chain joining region (Jh) probe, a rearranged fragment at approximately 14 kb was detected (data not shown). When reprobed with either of two different IL-3 probes, a rearranged 14 kb fragment, comigrating with the rearranged Jh fragment, was identified. When leukemic DNA was digested with *Hin*dIII plus *Eco*RI, a rearranged Jh fragment was detected at 6 kb. The IL-3 probes also identified a comigrating fragment of this size. These experiments indicated that the leukemic sample studied was clonal and that a single fragment contained both Jh and IL-3 sequences, suggesting a translocation had occurred.

To characterize better the joining of the IL-3 gene and the immunoglobulin heavy chain (IgH) gene, the polymerase chain reaction (PCR) was used to clone the translocation.[13] A Jh primer and an IL-3 primer were designed to produce an amplified product in the event of a head-to-head translocation. While control DNA gave no PCR product, Case 2 DNA yielded a PCR-derived fragment of approximately 980 bp, which was cloned and sequenced.

The DNA sequence of the translocation clone from Case 2 confirmed the joining of the Jh region with the promotor of the IL-3 gene in a head-to-head configuration (Fig 1). Sequence analysis indicated that the breakpoint on chromosome 14 was just upstream of the Jh5 coding region. The breakpoint on chromosome 5 occurred 934 bp upstream of the putative site of transcription initiation of the IL-3 gene. We also determined that a putative N sequence of 17 bp was inserted between the chromosome 5 and chromosome 14 sequences during the translocation event.[17,18] Figure 2 shows

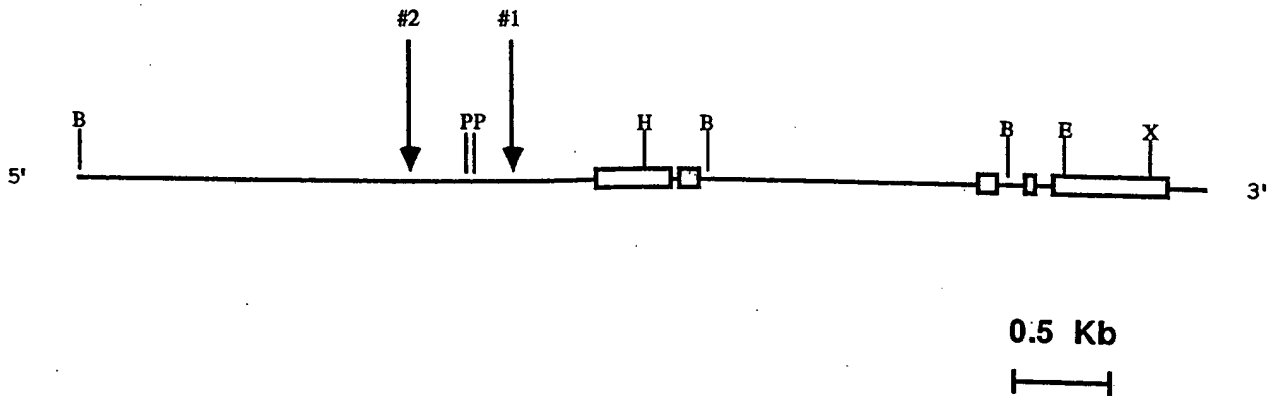

Fig 2. Relationship of chromosome 5 breakpoints to the IL-3 gene. This figure shows the two cloned breakpoints (arrows) in relation to the normal IL-3 gene.[8,10] One breakpoint occurred at position −462 and the other at −934 (arrows). In both circumstances, the translocations resulted in a head-to-head joining of the IgH gene and the IL-3 gene, leaving the mRNA and protein coding regions of the IL-3 gene intact. Boxes denote the five IL-3 exons; restriction enzymes are (B) *Bam*HI, (P) *Pst* I, (H) *Hpa* I, (E) *Eco*RI, and (X) *Xho* I.

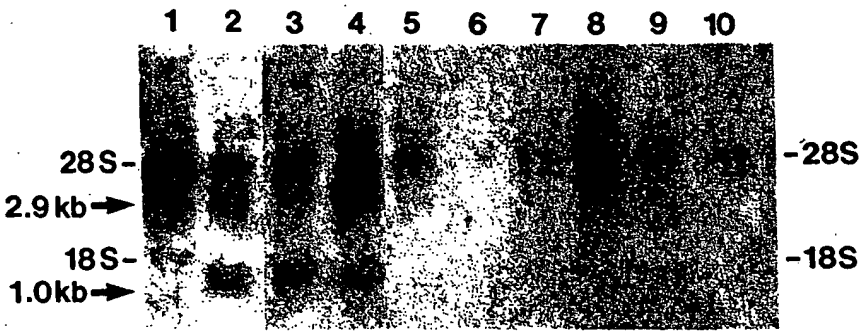

Fig 3. Documentation of IL-3 mRNA over-expression. A Northern blot was prepared and hybridized with a probe for IL-3. Lane 1 contained RNA from unstimulated peripheral blood lymphocytes (PBL) as a negative control. Lane 2 contained RNA from PBL stimulated for 4 hours with concanavalian A (ConA), and lane 3 contained RNA from PBL stimulated with ConA for 48 hours. As in the positive control lanes (2 and 3), a 1 kb band was identified in the leukemic sample from Case 1 (lane 4, lower arrow), suggesting aberrant expression of the IL-3 gene. In addition, the leukemic sample showed over-expression of an unspliced 2.9 kb IL-3 transcript (lane 4, upper arrow). We documented that this represented an unspliced precurser of the mature 1 kb transcript by showing that this band hybridized to a probe from intron 2 of the IL-3 gene. A similar 2.9 kb band was detected in lane 2, suggesting that an IL-3 mRNA of this size is sometimes detectable in normal mitogen-stimulated cells. Lane 5 through 10 represent RNA from six samples of B-lineage acute lymphocytic leukemia without the t(5;14) translocation, indicating that only the sample with the translocation exhibited IL-3 over-expression. Case 2 could not be analyzed by Northern blot because too few cells were available for study.

the locations of the two cloned breakpoints in relation to the IL-3 gene. The two chromosome 5 breakpoints were separated by less than 500 bp.

The genomic structure in Cases 1 and 2 suggested that a normal IL-3 gene product was over-expressed as a result of the altered promotor structure. This would predict that the IL-3 gene on the translocated chromosome was capable of making IL-3 protein. This prediction was tested by expressing a genomic fragment from the translocated allele of Case 1 containing all five IL-3 exons under the control of the SV40 promotor/enhancer in the Cos7 cell line. Cell supernatants were studied in a proliferation assay using the factor dependent erythroleukemic cell line, TF-1. The supernatants derived from transfections using the vector plus insert supported TF-1 proliferation, while supernatants from transfections using the vector alone were negative in this assay (data not shown). Furthermore, the biologic activity could be blocked by an antibody to human IL-3 (BVD3-6G8). This result showed that the translocated allele retained the ability to make IL-3 mRNA and protein.

The level of expression of IL-3 mRNA in leukemic cells from Case 1 was assessed. Northern blotting showed that the mature IL-3 mRNA (approximately 1 kb) and a 2.9 kb unspliced IL-3 mRNA were excessively produced by the leukemia (Fig 3). The 2.9 kb form of the mRNA is also present at low levels in normal peripheral blood T lymphocytes after mitogen activation (Fig 3). Several B-lineage acute leukemia samples without the t(5;14) translocation had undetectable levels of IL-3 mRNA in these experiments. In addition, although genes for GM-CSF and IL-5 map close to the IL-3 gene and might have been deregulated by the translocation, no IL-5 or GM-CSF mRNA could be detected in the leukemic sample (data not shown).[19,20]

Three serum samples from Case 2 were assayed by immunoassay for levels of IL-3, GM-CSF, and IL-5 (Table 1). Serum IL-3 could be detected and correlated with the clinical course. When the patient's leukemic cell burden was highest, the IL-3 level was highest. No serum GM-CSF or IL-5 could be detected.

Since the IL-3 immunoassay measured only immunoreactive factor, we confimed that biologically active IL-3 was present by using the TF-1 bioassay. This bioassay can be rendered monospecific using appropriate neutralizing monoclonal antibodies specific for IL-3, IL-5, or GM-CSF. We observed that sera from 1-16-84 and 3-14-84 contained TF-1 stimulating activity that could be blocked with anti-IL-3 MoAb (BVD3-6G8), but not with MoAbs to IL-5 (JES1-39D10) or GM-CSF (BVD2-23B6) (Fig 4; GM-CSF data not shown). The amount of neutralizable bioactivity in these two samples correlated very well with the difference in IL-3 levels obtained by immunoassay for these samples. Furthermore, the failure to block TF-1 proliferating activity with either anti-IL-5 or anti-GM-CSF was consistent with the inability to measure these factors by immunoassay and

Table 1. Peripheral Blood Counts and Growth Factor Levels at Different Times in Case 2

	Sample Date		
	11/15/83	1/16/84	3/14/84
Peripheral blood counts (cells/μL)			
WBC	81,800	116,500	12,300
Lymphoblasts	0	33,785	0
Eosinophils	46,626	73,080	615
Serum growth factor levels (pg/mL)			
IL-3	<444	7,995	1,051
GM-CSF	<15	<15	<15
IL-5	<50	<50	<50

Peripheral blood counts from Case 2 at three different time points with the corresponding growth factor levels quantified by immunoassay. The patient received chemotherapy between 1/16/84 and 3/14/84 to lower his leukemic burden.[3] No serum samples were available for a similar analysis of Case 1.

Abbreviation: WBC, white blood cells.

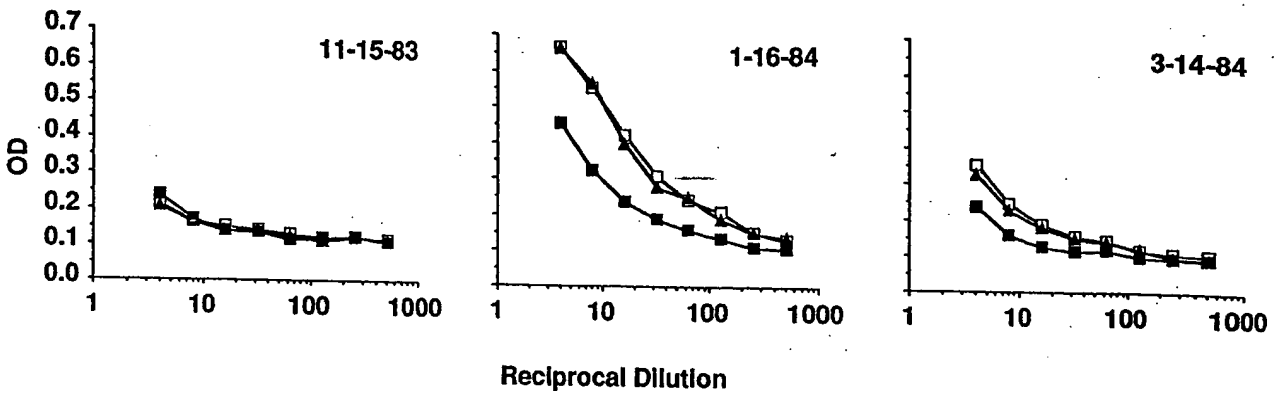

Fig 4. Bioassay of serum IL-3. Leukemic patient sera were tested for bioactive IL-3 and IL-5 in the TF-1 proliferation assay. The reciprocal of the dilution is indicated on the horizontal axis and the optical density indicating the amount of proliferation is indicated on the vertical axis. Serum from all three time points was assayed simultaneously. The assay was rendered monospecific by using a 1 μg/mL final concentration of monoclonal rat anti–IL-3, BVD3-6G8 (■), or anti–IL-5, JES1-39D10 (▲); □ indicates no MoAb. On 1/16/84 and 3/14/84, inhibition of proliferation was evident in the presence of anti–IL-3 antibody, documenting serum levels of IL-3 on those days. Serum IL-5 was not detected in this assay, as anti–IL-5 did not alter TF-1 proliferation.

indicated that these other myeloid growth factors were not detectably circulating in the serum of this patient.

DISCUSSION

In this report, we have extended our analysis of acute lymphocytic leukemia and eosinophilia associated with the t(5;14) translocation. In both cases we have studied, we have documented the joining of the IL-3 gene from chromosome 5 to the IgH gene from chromosome 14. The breakpoints on chromosome 5 are within 500 bp of each other, suggesting that additional breakpoints will be clustered in a small region of the IL-3 promotor. The PCR assay we have developed will be useful in the screening of additional clinical samples for this abnormality.

The finding of a disrupted IL-3 promotor associated with an otherwise normal IL-3 gene implied that this translocation might lead to the over-expression of a normal IL-3 gene product. In this work, we have documented that this is true. In addition, neither GM-CSF nor IL-5 are over-expressed by the leukemic cells. Furthermore, in one patient, serum IL-3 could be measured and correlated with disease activity. To our knowledge, this is the first measurement of human IL-3 in serum and its association with a disease process. The measurement of serum IL-3 in this and other clinical settings may now be indicated.

The finding of the IL-3 gene adjacent to a cancer-associated translocation breakpoint suggests that its activation is important for oncogenesis. It is our thesis that an autocrine loop for IL-3 is important for the evolution of this leukemia.[21] The excessive IL-3 production that we have documented would be one feature of such an autocrine loop. The final proof of our thesis must await additional data. In particular, from the study of additional clinical samples, it will be necessary to document that the IL-3 receptor is present on the leukemic cells and that anti–IL-3 antibody decreases proliferation of the leukemia in vitro.

An important aspect of this work is the suggestion of a therapeutic approach for this disease. If an autocrine loop for IL-3 can be documented in this disease, attempts to lower circulating IL-3 levels or block the interaction of IL-3 with its receptor may prove useful. Because it is also possible that the eosinophilia in these patients is mediated by the paracrine effects of leukemia-derived IL-3, similar interventions may improve this aspect of the disease. Antibodies or engineered ligands to accomplish these goals may soon be available.

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Clinical and Pathologic Significance of the c-*erb*B-2 (*HER*-2/*neu*) Oncogene

Timothy P. Singleton and John G. Strickler

The c-*erb*B-2 oncogene was first shown to have clinical significance in 1987 by Slamon et al,[79] who reported that c-*erb*B-2 DNA amplification in breast carcinomas correlated with decreased survival in patients with metastasis to axillary lymph nodes. Subsequent studies, however, of c-*erb*B-2 activation in breast carcinoma reached conflicting conclusions about its clinical significance. This oncogene also has been reported to have clinical and pathologic implications in other neoplasms. Our review summarizes these various studies and examines the clinical relevance of c-*erb*B-2 activation, which has not been emphasized in recent reviews.[37,38,55] The molecular biology of the c-*erb*B-2 oncogene has been extensively reviewed[37,38,55] and will be discussed only briefly here.

BACKGROUND

The c-*erb*B-2 oncogene was discovered in the 1980s by three lines of investigation. The *neu* oncogene was detected as a mutated transforming gene in neuroblastomas induced by ethylnitrosurea treatment of fetal rats.[6,73,74,78] The c-*erb*B-2 was a human gene discovered by its homology to the retroviral gene v-*erb*B.[33,49,76] *HER*-2 was isolated by screening a human genomic DNA library for homology with v-*erb*B.[24] When the DNA sequences were determined subsequently, c-*erb*B-2, *HER*-2, and *neu* were found to represent the same gene. Recently, the c-*erb*B-2 oncogene also has been referred to as *NGL*.

The c-*erb*B-2 DNA is located on human chromosome 17q21[24,33,66] and codes for c-*erb*B-2 mRNA (4.6 kb), which translates c-*erb*B-2 protein (p185). This

protein is a normal component of cytoplasmic membranes. The c-*erb*B-2 oncogene is homologous with, but not identical to, c-*erb*B-1, which is located on chromosome 7 and codes for the epidermal growth factor receptor.[8,103] The c-*erb*B-2 protein is a receptor on cell membranes and has intracellular tyrosine kinase activity and an extracellular binding domain.[2,105] Electron microscopy with a polyclonal antibody detects c-*erb*B-2 immunoreactivity on cytoplasmic membranes of neoplasms, especially on microvilli and the non-villous outer cell membrane.[61] In normal cells, immunohistochemical reactivity for c-*erb*B-2 is frequently present at the basolateral membrane or the cytoplasmic membrane's brush border.[22,62]

There is experimental evidence that c-*erb*B-2 protein may be involved in the pathogenesis of breast neoplasia. Overproduction of otherwise normal c-*erb*B-2 protein can transform a cell line into a malignant phenotype.[25] Also, when the *neu* oncogene containing an activating point mutation is placed in transgenic mice with a strong promoter for increased expression, the mice develop multiple independent mammary adenocarcinomas.[16,63] In other experiments, monoclonal antibodies against the *neu* protein inhibit the growth (in nude mice) of a *neu*-transformed cell line,[26–28] and immunization of mice with *neu* protein protects them from subsequent tumor challenge with the *neu*-transformed cell line.[14] Some authors have speculated that the use of antagonists for the unknown ligand could be useful in future chemotherapy.[85] Further review of this experimental evidence is beyond the scope of this article.

The c-*erb*B-2 activation most likely occurs at an early stage of neoplastic development. This hypothesis is supported by the presence of c-*erb*B-2 activation in both in situ and invasive breast carcinomas. In addition, studies of metastatic breast carcinomas usually demonstrate uniform c-*erb*B-2 activation at multiple sites in the same patient,[11,12,39,41,52] although c-*erb*B-2 activation has rarely been detected in metastatic lesions but not in the primary tumor.[57,60,107] Even more rarely, c-*erb*B-2 DNA amplification has been detected in a primary breast carcinoma but not in its lymph node metastasis.[5] In patients who have bilateral breast neoplasms, both lesions have similar patterns of c-*erb*B-2 activation, but only a few such cases have been studied.[11]

MECHANISMS OF c-*erb*B-2 ACTIVATION

The most common mechanism of c-*erb*B-2 activation is genomic DNA amplification, which almost always results in overproduction of c-*erb*B-2 mRNA and protein.[17,34,65,81] The c-*erb*B-2 amplification may stabilize the overproduction of mRNA or protein through unknown mechanisms. Human breast carcinomas with c-*erb*B-2 amplification contain 2 to 40 times more c-*erb*B-2 DNA[4,5] and 4 to 128 times more c-*erb*B-2 mRNA[34,90] than found in normal tissue. Most human breast carcinomas with c-*erb*B-2 amplification have 2 to 15 times more c-*erb*B-2 DNA. Tumors with greater amplification tend to have greater overproduction.[17,52,65] The non-mammary neoplasms that have been studied tend to have

similar levels of c-*erb*B-2 amplification or overproduction relative to the corresponding normal tissue.

The second most common mechanism of c-*erb*B-2 activation is overproduction of c-*erb*B-2 mRNA and protein without amplification of c-*erb*B-2 DNA.[81] The quantities of mRNA and protein usually are less than those in amplified cases and may approach the small quantities present in normal breast or other tissues.[17,50,52] The c-*erb*B-2 protein overproduction without mRNA overproduction or DNA amplification has been described in a few human breast carcinoma cell lines.[47]

Other rare mechanisms of c-*erb*B-2 activation have been reported. Translocations involving the c-*erb*B-2 gene have been described in a few mammary and gastric carcinomas, although some reported cases may represent restriction fragment length polymorphisms or incomplete restriction enzyme digestions that mimic translocations.[31,65,75,84,90,108] A single point mutation in the transmembrane portion of *neu* has been described in rat neuroblastomas induced by ethylnitrosurea.[9,55] The mutated *neu* protein has increased tyrosine kinase activity and aggregates at the cell membrane.[10,83,98] Although there has been speculation that some of the amplified c-*erb*B-2 genes may contain point mutations,[46] none has been detected in primary human neoplasms.[41,53,61]

TECHNIQUES FOR DETECTING c-*erb*B-2 ACTIVATION

Detection of c-*erb*B-2 DNA Amplification

Amplification of c-*erb*B-2 DNA is usually detected by DNA dot blot or Southern blot hybridization. In the dot blot method, the extracted DNA is placed directly on a nylon membrane and hybridized with a c-*erb*B-2 DNA probe. In the Southern blot method, the extracted DNA is treated with a restriction enzyme, and the fragments are separated by electrophoresis, transferred to a nylon membrane, and hybridized with a c-*erb*B-2 DNA probe. In both techniques, c-*erb*B-2 amplification is quantified by comparing the intensity (measured by densitometry) of the hybridization bands from the sample with those from control tissue.

Several technical problems may complicate the measurement of c-*erb*B-2 DNA amplification. First, the extracted tumor DNA may be excessively degraded or diluted by DNA from stromal cells.[81] Second, the c-*erb*B-2 DNA probe must be carefully chosen and labeled. For example, oligonucleotide c-*erb*B-2 probes may not be sensitive enough for measuring a low level of c-*erb*B-2 amplification, because diploid copy numbers can be difficult to detect (unpublished data). Third, the total amounts of DNA in the sample and control tissue must be compensated for, often with a probe to an unamplified gene. Many studies have used control probes to genes on chromosome 17, the location of c-*erb*B-2, to correct for possible alterations in chromosome number. Identical results, however, are obtained by using control probes to genes on other chromosomes,[5,65,80] with rare exception.[17] Studies using control probes to the beta-

globin gene must be interpreted with caution, because one allele of this gene is deleted occasionally in breast carcinomas.[3]

Amplification of c-*erb*B-2 DNA was assessed by using the polymerase chain reaction (PCR) in one recent study.[32] Oligoprimers for the c-*erb*B-2 gene and a control gene are added to the sample's DNA, and PCR is performed. If the sample contains more copies of c-*erb*B-2 DNA than of the control gene, the c-*erb*B-2 DNA is replicated preferentially.

Detection of c-*erb*B-2 mRNA Overproduction

Overproduction of c-*erb*B-2 mRNA usually is measured by RNA dot blot or Northern blot hybridization. Both techniques require extraction of RNA but otherwise are analogous to DNA dot blot and Southern blot hybridization. Use of PCR for detection of c-*erb*B-2 mRNA has been described in two recent abstracts.[89,102]

Overproduction of c-*erb*B-2 mRNA can be measured by in situ hybridization. Sections are mounted on glass slides, treated with protease, hybridized with a radiolabeled probe, washed, treated with nuclease to remove unbound probe, and developed for autoradiography. Silver grains are seen only over tumor cells that overproduce c-*erb*B-2 mRNA. Negative control probes are used.[65,96,106] Our experience indicates that these techniques are relatively insensitive for detecting c-*erb*B-2 mRNA overproduction in routinely processed tissue. Although the sensitivity may be increased by modifications that allow simultaneous detection of c-*erb*B-2 DNA and mRNA, in situ hybridization still is cumbersome and expensive (unpublished data).

All of the above c-*erb*B-2 mRNA detection techniques have several problems that make them more difficult to perform than techniques for detecting DNA amplification. One major problem is the rapid degradation of RNA in tissue that is not immediately frozen or fixed. In addition, during the detection procedure, RNA can be degraded by RNase, a ubiquitous enzyme, which must be eliminated meticulously from laboratory solutions. Third, control probes to genes that are uniformly expressed in the tissue of interest need to be carefully selected.

Detection of c-*erb*B-2 Protein Overproduction

The most accurate methods for detecting c-*erb*B-2 protein overproduction are the Western blot method and immunoprecipitation. Both techniques can document the binding specificity of various antibodies against c-*erb*B-2 protein. In Western blot studies, protein is extracted from the tissue, separated by electrophoresis (according to size), transferred to a membrane, and detected by using antibodies to c-*erb*B-2. In immunoprecipitation studies, antibodies against c-*erb*B-2 are added to a tumor lysate, and the resulting protein–antibody precipitate is separated by gel electrophoresis and stained for protein. Both Western blot and immunoprecipitation are useful research tools but currently are not practical for diagnostic pathology. Two recent abstracts have described an enzyme-linked immunosorbent assay (ELISA) for detection of c-*erb*B-2 protein.[19,45]

Overproduction of c-*erb*B-2 protein is most commonly assessed by various immunohistochemical techniques. These procedures often generate conflicting results, which are explained at least partially by three factors. First, various studies have used different polyclonal and monoclonal antibodies. Because some polyclonal antibodies recognize weak bands in addition to the c-*erb*B-2 protein band on Western blot or immunoprecipitation, the results of these studies should be interpreted with caution.[22,36,47,61] Even some monoclonal antibodies immunoprecipitate protein bands in addition to c-*erb*B-2 (p185).[30,59,66] Second, tissue fixation contributes to variability between studies. For example, some antibodies detect c-*erb*B-2 protein only in frozen tissue and do not react in fixed tissue. In general, formalin fixation diminishes the sensitivity of immunohistochemical methods and decreases the number of reactive cells.[61,66] When Bouin's fixative is used, there may be a higher percentage of positive cases.[22] Third, minimal criteria for interpreting immunohistochemical staining are generally lacking. Although there is general agreement that distinct crisp cytoplasmic membrane staining is diagnostic for c-*erb*B-2 activation in breast carcinoma, the number of positive cells and the staining intensity required to diagnose c-*erb*B-2 protein overproduction varies from study to study and from antibody to antibody. Degradation of c-*erb*B-2 protein is not a problem because it can be detected in intact form more than 24 hours after tumor resection without fixation or freezing.[64]

ACTIVATION OF c-*erb*B-2 IN BREAST LESIONS

Incidence of c-*erb*B-2 Activation

Most studies of c-*erb*B-2 oncogene activation do not specify histological subtypes of infiltrating breast carcinoma. Amplification of c-*erb*B-2 DNA was found in 19.1 percent (519 of 2715) of invasive carcinomas in 25 studies (Table 1), and c-*erb*B-2 mRNA or protein overproduction was detected in 20.9 percent (566 of 2714) of invasive carcinomas in 20 studies. Twelve studies have documented c-*erb*B-2 mRNA or protein overproduction in 15 percent (88 of 604) of carcinomas that lacked c-*erb*B-2 DNA amplification.

The incidence of c-*erb*B-2 activation in infiltrating breast carcinoma varies with the histological subtype. Approximately 22 percent (142 of 650) of infiltrating ductal carcinomas have c-*erb*B-2 activation, as expected from the above data. Other variants of breast carcinoma with frequent c-*erb*B-2 activation are inflammatory carcinoma (62 percent, 54 of 87), Paget's disease (82 percent, 9 of 11), and medullary carcinoma (22 percent, 5 of 23). In contrast, c-*erb*B-2 activation is infrequent in infiltrating lobular carcinoma (7 percent, 5 of 73) and tubular carcinoma (7 percent, 1 of 15).

The c-*erb*B-2 protein overproduction is present in 44 percent (44 of 100) of ductal carcinomas in situ and especially comedocarcinoma in situ (68 percent, 49 of 72). The micropapillary type of ductal carcinoma in situ also tends to have c-*erb*B-2 activation,[40,54,68] especially if larger cells are present. The greater fre-

TABLE 1. c-*erb*B-2 ACTIVATION IN MALIGNANT HUMAN BREAST NEOPLASMS

Histological Diagnosis[a]	c-*erb*B-2 DNA Amplification[a]	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Overproduction[b]
Carcinoma, not otherwise specified	146/526,[81] 52/310,[17] 52/291,[109] 28/176,[87] 17/157,[113] 22/141,[35] 14/136,[57] 12/122,[4] 19/103,[79] 15/95,[90] 15/86,[111] 17/73,[77] 16/66,[42] 6/61,[50] 11/57,[82] 10/57,[85] 13/51,[13] 8/49,[21] 10/38,[22] 12/36,[94] 1/25,[15] 7/24,[91] 7/15,[31] 7/10,[98] 2/10[107]	42/180,[69] 49/126,[35] 19/62,[65] 19/57,[50] 3/11,[90] 6/10,[96] 3/9[31]	118/728,[85b] 58/330,[17b] 47/313,[88] 17/195,[11] 32/191,[58] 31/185,[101] 34/102,[42] 24/53,[52b] 23/47,[13] 22/45,[8] 11/36,[94] 7/24,[91] 1/10[81]
Carcinoma, type not specified but lacking c-*erb*B-2 DNA amplification	—	18/136,[81] 14/73,[34] 8/16,[65] 0/8,[90] 1/4,[31] 0/3[96]	16/231,[17b] 18/136,[81] 13/35,[13] 14/29,[52b] 1/28,[92] 3/24,[94] 0/17[91]
Infiltrating ductal carcinoma	21/118,[92] 23/107,[34] 17/50,[44] 7/37[93] 14/53 (comedo-carcinoma)[16] 3/33 (tubuloductal carcinoma)[16]	35/95[24]	22/137,[40] 14/93,[39] 9/34[88]

Inflammatory carcinoma	33/80,[35] 3/6[52]	46/75[35]	5/6[52b]
Paget's disease	—	—	5/6,[40] 2/3,[54] 2/2[92]
Tubular carcinoma	0/5,[16] 0/1[93]	—	1/9[40]
Medullary carcinoma	2/4,[16] 0/1[34]	0/1[34]	1/12,[40] 1/3,[68] 1/2,[92] 0/1[39]
Mucinous carcinoma	0/1,[16] 0/1[93]	—	1/2[68]
Invasive papillary carcinoma	0/2[93]	—	—
Infiltrating lobular carcinoma	1/15,[16] 0/6[34]	1/5[34]	2/27,[92] 0/12,[40] 0/9,[39] 1/5[68]
Mammary fibrosarcoma	0/1[93]	—	—
"Benign cystosarcoma"	—	—	0/1[68]
Ductal CIS[c] with minimal invasion	3/5[92]	—	—
Ductal CIS	0/2[34]	1/2[34]	33/74,[40] 10/24[39]
Ductal CIS, solid or comedo type	—	—	20/33,[68] 19/29,[92] 10/10[54]
Ductal CIS, micropapillary type	—	—	10/10[68]
Ductal CIS, micropapillary or cribriform type	—	—	1(focal)/14[54]
Ductal CIS, papillary or cribriform type	—	—	0/16,[92] 1/9,[68] 0/3[40]
Lobular CIS	—	—	0/16[40]

[a]Shown as number of cases with activation/number of cases studied; reference is given as a superscript.
[b]These protein studies used Western blots; the rest used immunohistochemical methods.
[c]CIS = carcinoma in situ.

quency of c-*erb*B-2 protein overproduction in comedocarcinoma in situ, compared with infiltrating ductal carcinoma, could be explained by the fact that many infiltrating ductal carcinomas arise from other types of intraductal carcinoma, which show c-*erb*B-2 activation infrequently. Others have speculated that carcinoma in situ with c-*erb*B-2 activation tends to regress or to lose c-*erb*B-2 activation during progression to invasion.[40,68,92] Infiltrating and in situ components of ductal carcinoma, however, usually are similar with respect to c-*erb*B-2 activation,[11,39] although some authors have noted more heterogeneity of the immunohistochemical staining pattern in invasive than in in situ carcinoma.[40,42,88] Activation of c-*erb*B-2 is infrequent in lobular carcinoma in situ. If lesions contain more than one histological pattern of carcinoma in situ, the c-*erb*B-2 protein overproduction tends to occur in the comedocarcinoma in situ but may include other areas of carcinoma in situ.[42,54,68] Overproduction of c-*erb*B-2 protein in ductal carcinoma in situ correlates with larger cell size and a periductal lymphoid infiltrate.[68]

Activation of c-*erb*B-2 has not been identified in benign breast lesions, including fibrocystic disease, fibroadenomas, and radial scars (Table 2). Strong membrane immunohistochemical reactivity for c-*erb*B-2 has not been described in atypical ductal hyperplasia, although weak accentuation of membrane staining has been noted infrequently.[39,42,54] In normal breast tissue, c-*erb*B-2 DNA is diploid, and c-*erb*B-2 is expressed at lower levels than in activated tumors.[34,35,65,88]

These preliminary data suggest that c-*erb*B-2 activation may not be useful for resolving many of the common problems in diagnostic surgical pathology. For example, c-*erb*B-2 activation is infrequent in tubular carcinoma and radial scars. In addition, because c-*erb*B-2 activation is unusual in atypical ductal hyperplasia, cribriform carcinoma in situ, and papillary carcinoma in situ, detection of c-*erb*B-2 activation in these lesions may not be helpful in their differential diagnosis. The histological features of comedocarcinoma in situ, which commonly overproduces c-*erb*B-2, are unlikely to be mistaken for those of benign lesions. Activation of

TABLE 2. c-*erb*B-2 ACTIVATION IN BENIGN HUMAN BREAST LESIONS

Histological Diagnosis	c-*erb*B-2 DNA Amplification[a]	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Overproduction
Fibrocystic disease	0/10[93]	—	0/32,[39] 0/9,[88] 0/8[88]
Atypical ductal hyperplasia	—	—	2(weak)/21,[54] 1(cytoplasmic)/13[39]
Benign ductal hyperplasia	—	—	0/12[39]
Sclerosing adenosis	—	—	0/4[39]
Fibroadenomas	0/16,[34] 0/6,[93] 0/2,[21] 0/1[91]	0/6,[35] 0/3[34]	0/21,[39] 0/10,[68] 0/8,[88] 0/3[42]
Radial scars	—	—	0/22[39]
Blunt duct adenosis	—	—	0/14[39]
"Breast mastosis"	—	0/3[35]	—

[a]Shown as number of cases with activation/number of cases studied; reference is given as a superscript.

c-*erb*B-2, however, does favor infiltrating ductal carcinoma over infiltrating lobular carcinoma. Further studies of these issues would be useful.

Correlation of c-*erb*B-2 Activation With Pathologic Prognostic Factors

Multiple studies have attempted to correlate c-*erb*B-2 activation with various pathologic prognostic factors (Table 3). Activation of c-*erb*B-2 was correlated with lymph node metastasis in 8 of 28 series, with higher histological grade in 6 of 17 series, and with higher stage in 4 of 14 series. Large tumor size was not associated with c-*erb*B-2 activation in most studies (11 of 14). Tetraploid DNA content and low proliferation, measured by Ki-67, have been suggested as prognostic factors and may correlate with c-*erb*B-2 activation.[6,7]

Correlation of c-*erb*B-2 Activation With Clinical Prognostic Factors

Various studies have attempted also to correlate c-*erb*B-2 activation with clinical features that may predict a poor outcome (Table 4). Activation of c-*erb*B-2 correlated with absence of estrogen receptors in 10 of 28 series and with absence of progesterone receptors in 6 of 18 series. In most studies, patient age did not correlate with c-*erb*B-2 activation, and, in the rest of the reports, c-*erb*B-2 activation was associated with either younger or older ages.

Correlation of c-*erb*B-2 Activation With Patient Outcome

Slamon et al[79,81] first showed that amplification of the c-*erb*B-2 oncogene independently predicts decreased survival of patients with breast carcinoma. The correlation of c-*erb*B-2 amplification with poor outcome was nearly as strong as the correlation of number of involved lymph nodes with poor outcome. Slamon et al also reported that c-*erb*B-2 amplification is an important prognostic indicator only in patients with lymph node metastasis.[79,81]

A large number of subsequent studies also attempted to correlate c-*erb*B-2 activation with prognosis (Table 5). In 12 series, there was a correlation between c-*erb*B-2 activation and tumor recurrence or decreased survival. In five of these series, the predictive value of c-*erb*B-2 activation was reported to be independent of other prognostic factors. In contrast, 18 series did not confirm the correlation of c-*erb*B-2 activation with recurrence or survival. Four possible explanations for this controversy are discussed below.

One problem is that c-*erb*B-2 amplification correlates with prognosis mainly in patients with lymph node metastasis. As summarized in Table 5, most studies of patients with axillary lymph node metastasis showed a correlation of c-*erb*B-2 activation with poor outcome. In contrast, most studies of patients without axillary metastasis have not demonstrated a correlation with patient outcome. Table 6 summarizes the studies in which all patients (with and without axillary metastasis) were considered as one group. There is a trend for studies with a higher percentage of metastatic cases to show an association between c-*erb*B-2 activation and poor outcome. Thus, most of the current evidence suggests that c-*erb*B-2 activation has prognostic value only in patients with metastasis to lymph nodes.

TABLE 3. CORRELATION OF c-*erb*B-2 ACTIVATION WITH PATHOLOGIC PROGNOSTIC FACTORS IN BREAST CARCINOMA

Prognostic Factor	P[a]	c-*erb*B-2 DNA Amplification[b]	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Overproduction[c]
Metastasis to axillary lymph nodes	<0.05	(118)[35] (105)[34] (49)[21]	(104)[35] (92)[24] (9)[81]	(350)[55c] (36)[13]
	0.05–0.15	(103)[79] (86)[79] (58)[111]	—	(189)[92]
	>0.15	(279)[17] (176)[67] (157)[113] (122)[4] (85)[90] (50)[52] (50)[44] (47)[13] (41)[93]	(50)[50]	(329)[17c] (261)[88] (195)[11] (185)[101] (102)[39] (50)[52b]
Larger size	<0.05	(280)[17]	—	(330)[17c] (189)[92]
	0.05–0.15	(86)[79]	—	—
	>0.15	(176)[67] (157)[113] (103)[79] (64)[77] (58)[111] (45)[21]	(51)[50]	(350)[55c] (185)[101] (34)[92]
Higher stage	<0.05	(300)[17] (64)[77] (58)[111]	—	(349)[17c]
	0.05–0.15	(56)[52]	—	—
	>0.15	(176)[67] (157)[113] (84)[90] (61)[60] (53)[21] (52)[67] (41)[93]	—	(102)[39] (56)[52b]
Higher histological grade	<0.05	(47)[13] (15)[31]	(53)[65]	(176)[101] (168)[11] (38)[13]
	0.05–0.15	—	—	—
	>0.15	(122)[4] (113)[34] (95)[90] (58)[111] (50)[44] (41)[93]	(86)[33] (65)[35]	(290)[88] (189)[92] (102)[39]

[a]A correlation is statistically significant at <0.05, equivocal at best between 0.05 and 0.15, and not statistically significant at >0.15.
[b]Numbers inside parentheses are the number of patients in an individual study; superscript is the reference. Some studies analyzed more than one group of patients.
[c]By Western blot method; all other protein studies used immunohistochemical methods.

TABLE 4. CORRELATION OF c-*erb*B-2 ACTIVATION WITH CLINICAL PROGNOSTIC FACTORS IN BREAST CARCINOMA

Prognostic Factor	P[a]	c-*erb*B-2 DNA Amplification[b]	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Overproduction[c]
Absence of estrogen receptors	<0.05	(253)[109] (141)[35] (109)[34] (86)[79] (50)[44] (47)[13]	(104)[35]	(350)[85c] (330)[17a] (185)[101]
	0.05–0.15	—	—	—
	>0.15	(157)[113] (122)[4] (103)[79] (95)[90] (64)[77] (61)[50] (58)[111] (53)[21] (51)[62] (41)[93]	(180)[89] (62)[65] (62)[85] (57)[50]	(290)[88] (172)[11] (51)[62c] (38)[13]
Absence of progesterone receptors	<0.05	(253)[109] (141)[35] (109)[34] (50)[44]	—	(350)[85c] (306)[17c]
	0.05–0.15	(86)[79] (49)[62]	—	—
	>0.15	(157)[113] (122)[4] (103)[79] (64)[77]	(180)[89] (103)[95] (62)[89] (56)[35]	(90)[11] (49)[62c]
Age (menopausal status)	<0.05	—	—	(younger: 330)[17c] (older: 56)[52c]
	0.05–0.15	(younger: 86)[79]	—	—
	>0.15	(230)[17] (176)[67] (157)[113] (122)[4] (116)[34] (103)[79] (95)[90] (64)[77] (58)[111] (56)[52] (53)[21] (49)[13] (41)[93] (15)[21]	(62)[65]	(350)[85c] (290)[88] (189)[92] (162)[11] (45)[92]

[a]A correlation is statistically significant at <0.05, equivocal at best between 0.05 and 0.15, and not statistically significant at >0.15
[b]Numbers inside parentheses are the number of patients in an individual study; superscript is the reference. Some studies analyzed more than one group of patients.
[c]By Western blot method; all other protein studies used immunohistochemical methods.

TABLE 5. CORRELATION OF c-*erb*B-2 ACTIVATION WITH OUTCOME IN PATIENTS WITH BREAST CARCINOMA

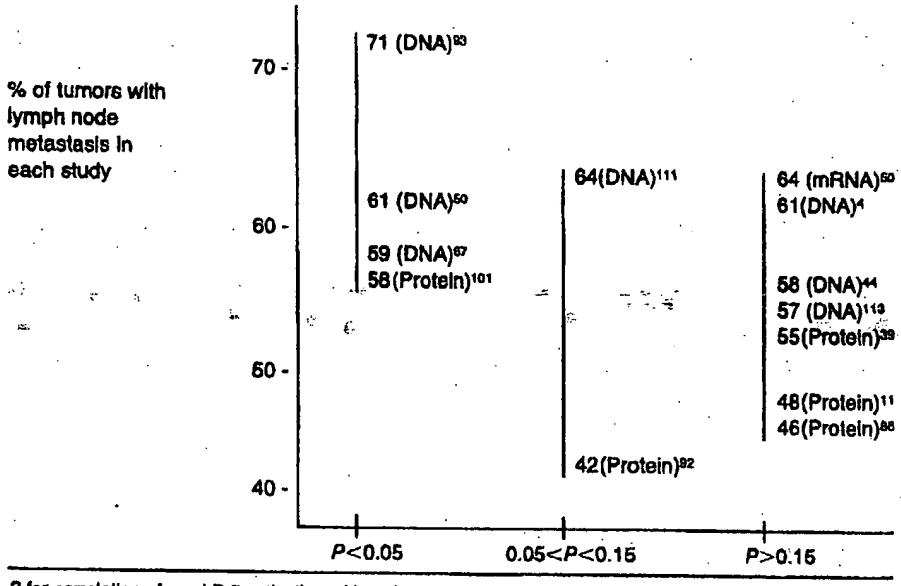
		Number of Patients				
P[a]	Type of c-*erb*B-2 Activation[b]	*Total*	*With Metastastis to Axillary Lymph Nodes*	*No Metastasis*	Statistical Analysis[c]	Reference
<0.05	DNA	176			M	87
<0.06	DNA	61			U	60
<0.05	DNA	57			U	65
<0.05	DNA	41			U	93
<0.05	mRNA	62			U	65
<0.05	Protein	102			M	101
<0.05	DNA		345		M	81
<0.05	DNA		120		U	17
<0.05	DNA		91		U	87
<0.05	DNA		86		M	79
<0.05	Protein-WB		350		M	85
<0.05	Protein		62	44	U	101
0.05–0.15	DNA	57			U	111
0.05–0.15	Protein	189			M	92
0.05–0.15	Protein		120		U	86
>0.15	DNA	130			U	113
>0.15	DNA	122			M	4
>0.15	DNA	50			U	44
>0.15	mRNA	57			U	50
>0.15	Protein	290			M	86
>0.15	Protein	195			U	11
>0.15	Protein	102			U	39
>0.15	Protein		137		U	17
>0.15	DNA			181	M	81
>0.15	DNA			159	U	17
>0.15	DNA			73	U	87
>0.15	Protein-WB			378	U	85
>0.15	Protein-WB			192	U	17
>0.15	Protein			141	U	86
>0.15	Protein			41	U	40

[a]The endpoints of these studies were tumor recurrence or decreased survival or both. Correlation between c-*erb*B-2 activation and a poorer patient outcome is statistically significant at <0.05, is of equivocal significance at 0.05 to 0.15, and is not significant at >0.15.

[b]Shown as variable measured. Letters "WB" indicate assay by Western blot; the other protein studies used immunohistochemical methods.

[c]M = multivariate statistical analysis; U = univariate statistical analysis.

TABLE 6. PERCENTAGE OF BREAST CARCINOMAS WITH METASTASIS COMPARED WITH PROGNOSTIC SIGNIFICANCE OF c-*erb*B-2 ACTIVATION



P for correlation of c-*erb*B-2 activation with patient outcome.
Each study's percentage of breast carcinomas with metastasis is compared with the correlation between c-*erb*B-2 activation and outcome. These data include only those studies that considered, as one group, all breast cancer patients, whether or not they had axillary metastasis. Superscripts are the references. In parentheses are the types of c-*erb*B-2 activation. *P* values are interpreted as in Table 3.

A second problem is that various types of breast carcinoma are grouped together in many survival studies. Because the current literature suggests that c-*erb*B-2 activation is infrequent in lobular carcinoma, studies that combine infiltrating ductal and lobular carcinomas may dilute the prognostic effect of c-*erb*B-2 activation in ductal tumors. In addition, most studies do not analyze inflammatory breast carcinoma separately. This condition frequently shows c-*erb*B-2 activation and has a worse prognosis than the usual mammary carcinoma, but it is an uncommon lesion.

A third potential problem is the paucity of studies that attempt to correlate c-*erb*B-2 activation with clinical outcome in subsets of breast carcinoma without metastasis. Two recent abstracts reported that in patients without lymph node metastasis who had various risk factors for recurrence (such as large tumor size and absence of estrogen receptors), c-*erb*B-2 overexpression predicted early recurrence.[23,67] In patients with ductal carcinoma in situ, one small study found no association between tumor recurrence and c-*erb*B-2 activation.[40]

A fourth problem is the lack of data regarding whether the prognosis correlates better with c-*erb*B-2 DNA amplification or with mRNA or protein overproduction. Most studies that find a correlation between c-*erb*B-2 activa-

tion and poor patient outcome measure c-*erb*B-2 DNA amplification (Table 5), and breast carcinoma patients with greater amplification of c-*erb*B-2 may have poorer survival.[78,81] Recent studies suggest that amplification has more prognostic power than overproduction,[17,34,35] but the clinical significance of c-*erb*B-2 overproduction without DNA amplification deserves further research.[17,52] Few studies have attempted to correlate patient outcome with c-*erb*B-2 mRNA overproduction, and many studies of c-*erb*B-2 protein overproduction use relatively less reliable methods such as immunohistochemical studies with polyclonal antibodies.

Comparison of c-*erb*B-2 Activation With Other Oncogenes in Breast Carcinoma

Other oncogenes that may have prognostic implications in human breast cancer are reviewed elsewhere.[71,106] This section will be restricted to a comparison between the clinical relevance of c-*erb*B-2 and these other oncogenes.

The c-*myc* gene is often activated in breast carcinomas, but c-*myc* activation generally has less prognostic importance than c-*erb*B-2 activation.[21,34,77,87,93] One study found a correlation between increased mRNAs of c-*erb*B-2 and c-*myc*, although other reports have not confirmed this.[34,106] Subsequent research, however, could demonstrate a subset of breast carcinomas in which c-*myc* has more prognostic importance than c-*erb*B-2.

The gene c-*erb*B-1 for the epidermal growth factor receptor (EGFR) is homologous with c-*erb*B-2 but is infrequently amplified in breast carcinomas.[79] Overproduction of EGFR, however, occurs more frequently than amplification and may correlate with a poor prognosis. In studies that have examined both c-*erb*B-2 and EGFR in the same tumor, c-*erb*B-2 has a stronger correlation with poor prognostic factors.[35,52] Studies have tended to show no correlation between amplification of c-*erb*B-2 and c-*erb*B-1 or overproduction of c-*erb*B-2 and EGFR, although at the molecular level EGFR mediates phosphorylation of c-*erb*B-2 protein.[51,52,61,68,109] Recent reviews describe EGFR in breast carcinoma.[43,100]

The genes c-*erb*A and *ear*-1 are homologous to the thyroid hormone receptor, and they are located adjacent to c-*erb*B-2 on chromosome 17. These genes are frequently coamplified with c-*erb*B-2 in breast carcinomas. The absence of c-*erb*A expression in breast carcinomas, however, is evidence against an important role for this gene in breast neoplasia.[90] Amplification of c-*erb*B-2 can occur without *ear*-1 amplification, and these tumors have a decreased survival that is similar to tumors with both c-*erb*B-2 and *ear*-1 amplification.[67] Consequently, c-*erb*B-2 amplification seems to be more important than amplification of c-*erb*A or *ear*-1.

Other genes also have been compared with c-*erb*B-2 activation in breast carcinomas. One study found a significant correlation between increased c-*erb*B-2 mRNA and increased mRNAs of *fos*, platelet-derived growth factor chain A, and Ki-*ras*.[106] Allelic deletion of c-Ha-*ras* may indicate a poorer prognosis in breast carcinoma,[21] but it has not been compared with c-*erb*B-2 activation. Some studies have suggested a correlation between advanced stage or recurrence of breast carcinoma and activation of any one of several oncogenes.[21,113]

ACTIVATION OF c-*erb*B-2 IN NON-MAMMARY TISSUES

Incidence of c-*erb*B-2 Activation in Non-Mammary Tissues

Table 7 summarizes the normal tissues in which c-*erb*B-2 expression has been detected, usually with immunohistochemical methods using polyclonal anti-

TABLE 7. PRESENCE OR ABSENCE OF c-*erb*B-2 mRNA OR c-*erb*B-2 PROTEIN IN NORMAL HUMAN TISSUES

Tissues With c-*erb*B-2 mRNA	Tissues Producing c-*erb*B-2 Protein[a]	Tissues Lacking c-*erb*B-2 mRNA	Tissues Lacking c-*erb*B-2 Protein
Skin[24]	Epidermis[56]		
	External root sheath[56]		
	Eccrine sweat gland[56]		
	Fetal oral mucosa[62]		Postnatal oral mucosa[62]
	Fetal esophagus[62]		Postnatal esophagus[62]
Stomach[24]	Stomach[22,62]		
	Fetal intestine[62a]		
Jejunum[24]	Small intestine[22,62]		
Colon[24]	Colon[22,62]		
Kidney[24]	Fetal kidney[62a]	Kidneys[104]	Glomerulus[62]
			Postnatal Bowman's capsule[62]
	Fetal proximal tubule[62]		Postnatal proximal tubule[62]
	Distal tubule[62]		
	Fetal collecting duct[62]		Postnatal collecting duct[62]
	Fetal renal pelvis[62]		Postnatal renal pelvis[62]
	Fetal ureter[62]		Postnatal fetal ureter[62]
Liver[24]	Hepatocytes[22]		Liver[62,95]
	Pancreatic acini[22]		
	Pancreatic ducts[22,62]		
	Endocrine cells of islets of Langerhans[22]		Pancreatic islets[62]
Lung[24]	Fetal trachea[62]		Postnatal trachea[62]
	Fetal bronchioles[62]		Postnatal bronchioles[62]
	Bronchioles[99]		
			Postnatal alveoli[62,99]
Fetal brain[24]			Postnatal brain[62]
	Fetal ganglion cells[62]		Postnatal ganglion cells[62]
Thyroid[1]			
Uterus[24]			
	Ovary[12]		
	Blood vessels[42]		Endothelium[62]
Placenta[24]			
			Adrenocortical cells[62]
			Postnatal thymus[62]
			Fibroblasts[62]
			Smooth muscle cells[62]
			Cardiac muscle cells[62]

[a]This protein study used Western blots; the rest used immunohistochemical methods.

bodies. Only a few studies have been performed, and some of these do not demonstrate convincing cell membrane reactivity in the published photographs. The interpretations in these studies, however, are listed, with the caveat that these findings should be confirmed by immunoprecipitation or Western or RNA blots. Production of c-*erb*B-2 has been identified in normal epithelium of the gastrointestinal tract and skin. Discrepancies regarding c-*erb*B-2 protein in other tissues could be due, at least in part, to differences in techniques.

The data on c-*erb*B-2 activation in various non-mammary neoplasms should be interpreted with caution, because only small numbers of tumors have been studied, usually by immunohistochemical methods using polyclonal antibodies. Studies using cell lines have been excluded, because cell culture can induce amplification and overexpression of other genes, although this has not been documented for c-*erb*B-2.

Activation of c-*erb*B-2 has been identified in 32 percent (64 of 203) of ovarian carcinomas in eight studies (Table 8). One abstract[45] stated that ovarian carcinomas contained significantly more c-*erb*B-2 protein than ovarian non-epithelial malignancies. Another report[81] showed that 12 percent of ovarian carcinomas had c-*erb*B-2 overproduction without amplification.

Activation of c-*erb*B-2 has been identified in 20 percent (40 of 198) of gastric adenocarcinomas in seven studies, including 33 percent (21 of 64) of

TABLE 8. c-*erb*B-2 ACTIVATION IN HUMAN GYNECOLOGIC TUMORS[a]

Tumor Type	c-*erb*B-2 DNA Amplification	c-*erb*B-2 mRNA Over-production	c-*erb*B-2 Protein Over-production
Ovary—carcinoma, not otherwise specified	31/120,[81] 1/11,[57] 0/5,[107] 0/5,[84] 0/3,[112] 0/2,[72] 0/1[110]	23/67[81]	23/73,[12] 36/72[51]
Ovary—serous (papillary) carcinoma	2/7,[110] 1/7,[112] 0/6[72]	—	—
Ovary—endometrioid carcinoma	0/3[110]	—	—
Ovary—mucinous carcinoma	1/2,[110] 0/1[72]	—	—
Ovary—clear cell carcinoma	0/2,[112] 0/1[72]	—	—
Ovary—mixed epithelial carcinoma	0/2[72]	—	—
Ovary—endometrioid borderline tumor	0/1[72]	—	—
Ovary—mucinous borderline tumor	0/3[72]	—	—
Ovary—serous cystadenoma	0/4[72]	—	—
Ovary—mucinous cystadenoma	0/2[72]	—	—
Ovary—sclerosing stromal tumor	0/1[72]	—	—
Ovary—fibrothecoma	0/1[72]	—	—
Uterus—endometrial adenocarcinoma	0/4,[84] 0/1[110]	—	—

[a]Shown as number of cases with amplification (or overproduction)/total number of cases studied; reference is given as superscript. All protein studies used immunohistochemical methods.

intestinal or tubular subtypes and 9 percent (4 of 47) of diffuse or signet ring cell subtypes (Table 9). Activation of c-*erb*B-2 has been detected in 2 percent (6 of 281) of colorectal carcinomas, although an additional immunohistochemical study detected c-*erb*B-2 protein in seven of eight tissues fixed in Bouin's solution. One study found greater immunohistochemical reactivity for c-*erb*B-2 protein in colonic adenomatous polyps than in the adjacent normal epithelium, using Bouin's fixative. Lesions with anaplastic features and progression to invasive carcinoma tended to show decreased immunohistochemical reactivity for c-*erb*B-2 protein.[22] Hepatocellular carcinomas (12 of 14 cases) and cholangiocarcinomas (46 of 63 cases) reacted with antibodies against c-*erb*B-2 in one study, but some of these "positive" cases showed only diffuse cytoplasmic staining, which

TABLE 9. c-*erb*B-2 ACTIVATION IN HUMAN GASTROINTESTINAL TUMORS[a]

Tumor Type	c-*erb*B-2 DNA Amplification	c-*erb*B-2 Protein Overproduction
Esophagus—squamous cell carcinoma	0/1[107]	0/1[61]
Stomach—carcinoma, poorly differentiated	0/22[108]	—
Stomach—adenocarcinoma	2/24,[94] 2/9,[107] 2/8,[111] 2/8,[57] 0/1[108]	4/27,[29] 3/10[61]
Stomach—carcinoma, intestinal or tubular type	5/10[108]	16/54[29]
Stomach—carcinoma, diffuse or signet ring cell type	0/2[108]	4/45[29]
Colorectum—carcinoma	2/49,[94] 1/45,[111] 1/45,[57] 1/45,[60] 0/40,[81] 0/32,[107] 0/3[82]	1/22,[58] 7/8[22b]
Colon—villous adenoma	0/1[60]	—
Colon—tubulovillous adenoma	0/5[60]	—
Colon—tubular adenoma	0/7[60]	19/19[22b]
Colon—hyperplastic polyp	0/1[60]	—
Intestine—leiomyosarcoma	—	0/1[61]
Hepatocellular carcinoma	0/12[111]	12/14,[95] 0/2[61]
Hepatoblastoma	0/1[57]	—
Cholangiocarcinoma	—	46/63[95]
Pancreas—adenocarcinoma	—	2/80,[41c] 0/2[61]
Pancreas—acinar carcinoma	—	0/1[41]
Pancreas—clear cell carcinoma	—	0/2[41]
Pancreas—large cell carcinoma	—	0/3[41]
Pancreas—signet ring carcinoma	—	0/1[41]
Pancreas—chronic inflammation	—	0/14[41c]

[a]Shown as number of cases with amplification (or overproduction)/total number of cases studied; reference is given as superscript. All protein studies used immunohistochemical methods. No studies analyzed for c-*erb*B-2 mRNA.

[b]Tissues fixed in Bouin's solution.

[c]Only cases with distinct membrane staining are interpreted as showing c-*erb*B-2 overproduction.

TABLE 10. c-*erb*B-2 ACTIVATION IN HUMAN PULMONARY TUMORS[a]

Tumor Type	c-*erb*B-2 DNA Amplification	c-*erb*B-2 Protein Overproduction
Non-small cell carcinoma	2/60,[75] 0/60[81]	1/84[59]
Epidermoid carcinoma	0/13,[62] 0/10,[57] 0/6[20]	3/5[59]
Adenocarcinoma	0/21,[62] 1/13,[20] 0/7,[111] 0/7,[57] 0/3[107]	4/12[99]
Large cell carcinoma	0/9,[62] 0/6[20]	—
Small cell carcinoma	—	0/26,[59] 0/3[99]
Carcinoid tumor	0/1[62]	0/3[99]

[a]Shown as number of cases with amplification (or overproduction)/total number of cases studied; reference is given as superscript. All protein studies used immunohistochemical methods. No studies analyzed for c-*erb*B-2 mRNA.

does not indicate c-*erb*B-2 activation in breast neoplasms.[95] Also, some pancreatic carcinomas and chronic pancreatitis tissue had cytoplasmic immunohistochemical reactivity for c-*erb*B-2 protein, in addition to the rare case of pancreatic adenocarcinoma with distinct cell membrane staining.[41]

Tables 10 through 14 summarize the studies of c-*erb*B-2 activation in other neoplasms. The c-*erb*B-2 oncogene is not activated in most of these tumors. Activation of c-*erb*B-2 has been detected in 1 percent (4 of 299) of pulmonary non-small cell carcinomas in nine studies, although one additional report[99] found c-*erb*B-2 protein overproduction in 41 percent (7 of 17). Renal cell carcinoma had c-*erb*B-2 activation in 7 percent (2 of 30) in four studies. Overproduction of c-*erb*B-2 protein was described in one transitional cell carcinoma of the urinary bladder, a grade 2 papillary lesion.[58] Squamous cell carcinoma and basal cell carcinoma of the skin may contain c-*erb*B-2 protein, but it is not clear

TABLE 11. c-*erb*B-2 ACTIVATION IN HUMAN HEMATOLOGIC PROLIFERATIONS[a]

Tumor Type	c-*erb*B-2 DNA Amplification	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Overproduction
Hematologic malignancies	0/23[111]	—	—
Malignant lymphoma	0/9,[57] 0/3[107]	0/1[1]	0/15[61]
Acute leukemia	0/14[57]	—	—
Acute lymphoblastic leukemia	0/1[107]	—	—
Acute myeloblastic leukemia	0/3[107]	—	—
Chronic leukemia	0/19[57]	—	—
Chronic lymphocytic leukemia	0/6[107]	—	—
Chronic myelogenous leukemia	0/6[107]	—	—
Myeloproliferative disorder	0/1[57]	—	—

[a]Shown as number of cases with amplification (or overproduction)/total number of cases studied; reference is given as superscript. All protein studies used immunohistochemical methods.

TABLE 12. c-*erb*B-2 ACTIVATION IN HUMAN TUMORS OF SOFT TISSUE AND BONE[a]

Tumor Type	c-*erb*B-2 DNA Amplification
Sarcoma	0/10,[111] 0/8[57]
Malignant fibrous histiocytoma	0/1[107]
Liposarcoma	0/3[107]
Pleomorphic sarcoma	0/1[107]
Rhabdomyosarcoma	0/1[107]
Osteogenic sarcoma	0/2,[107] 0/2[57]
Chondrosarcoma	0/1[107]
Ewing's sarcoma	0/1[57]
Schwannoma	0/1[57]

[a]Shown as number of cases with amplification (or overproduction)/total number of cases studied; reference is given as superscript. No studies analyzed for c-*erb*B-2 mRNA or c-*erb*B-2 protein.

whether the protein level is increased over that of normal skin.[56] Thyroid carcinomas and adenomas can have low levels of increased c-*erb*B-2 mRNA. One abstract described low-level c-*erb*B-2 DNA amplification in one of ten salivary gland pleomorphic adenomas.[48]

Correlation of c-*erb*B-2 Activation With Patient Outcome

Very few studies have attempted to correlate c-*erb*B-2 activation in non-mammary tumors with outcome. Slamon et al[61] showed that c-*erb*B-2 amplification or overexpression in ovarian carcinomas correlates with decreased survival, especially when marked activation is present. However, they did not report the stage, histological grade, or histological subtype of these neoplasms. Another study of stages III and IV ovarian carcinomas found a correlation between decreased survival and c-*erb*B-2 protein overproduction, but not between survival and histological grade.[12] One abstract stated that c-*erb*B-2 protein overproduction in 10 of 16 pulmonary adenocarcinomas correlated with decreased disease-free interval.[70] Another abstract described a tendency for immunohisto-

TABLE 13. c-*erb*B-2 ACTIVATION IN HUMAN TUMORS OF THE URINARY TRACT[a]

Tumor Type	c-*erb*B-2 DNA Amplification	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Overproduction
Kidney—renal cell carcinoma	1/5,[57] 1/4,[107] 0/5[84]	0/16[104]	—
Wilms' tumor	0/4[57]	—	—
Prostate—adenocarcinoma	—	—	0/23[58]
Urinary bladder—carcinoma	—	—	1/48[58]

[a]Shown as number of cases with amplification (or overproduction)/total number of cases studied; reference is given as superscript. All protein studies used immunohistochemical methods.

TABLE 14. c-*erb*B-2 ACTIVATION IN MISCELLANEOUS HUMAN TUMORS[a]

Tumor Type	c-*erb*B-2 DNA Amplification	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Over-production
Skin—malignant melanoma	—	—	0/10[58]
Skin, head and neck—squamous cell carcinoma	0/7[107]	—	—
Site not stated—squamous cell carcinoma	0/8,[57] 0/2[76]	—	—
Salivary gland—adenocarcinoma	1/1[76]	—	—
Parotid gland—adenoid cystic carcinoma	—	—	0/1[61]
Thyroid—anaplastic carcinoma	0/1[1]	0/1[1]	—
Thyroid—papillary carcinoma	0/5[1]	3(low levels)/5[1]	—
Thyroid—adenocarcinoma	0/1[84]	—	—
Thyroid—adenoma	0/2[1]	1(low levels)/2[1]	—
Neuroblastoma	0/35,[81] 0/9,[57] 0/1[76]	—	—
Meningioma	0/2[57]	—	—

[a]Shown as number of cases with amplification (or overproduction)/total number of cases studied; reference is given as superscript. All protein studies used immunohistochemical methods.

chemical reactivity for c-*erb*B-2 protein to correlate with higher grades of prostatic adenocarcinoma.[97] Additional prognostic studies of ovarian carcinomas and other neoplasms are needed.

SUMMARY

Activation of the c-*erb*B-2 oncogene can occur by amplification of c-*erb*B-2 DNA and by overproduction of c-*erb*B-2 mRNA and c-*erb*B-2 protein. Approximately 20 percent of breast carcinomas show evidence of c-*erb*B-2 activation, which correlates with a poor prognosis primarily in patients with metastasis to axillary lymph nodes. Studies that have attempted to correlate c-*erb*B-2 activation with other prognostic factors in breast carcinoma have reported conflicting conclusions. The pathologic and clinical significance of c-*erb*B-2 activation in other neoplasms is unclear and should be assessed by additional studies.

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DECLARATION OF PAUL POLAKIS, PH.D. UNDER 37 C.F.R. § 1.132

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Dear Sir:

Attached is the Declaration of Paul Polakis, Ph.D.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

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DECLARATION OF PAUL POLAKIS, Ph.D.

I, Paul Polakis, Ph.D., declare and say as follows:

1. I was awarded a Ph.D. by the Department of Biochemistry of the Michigan State University in 1984. My scientific Curriculum Vitae is attached to and forms part of this Declaration (Exhibit A).

2. I am currently employed by Genentech, Inc. where my job title is Staff Scientist. Since joining Genentech in 1999, one of my primary responsibilities has been leading Genentech's Tumor Antigen Project, which is a large research project with a primary focus on identifying tumor cell markers that find use as targets for both the diagnosis and treatment of cancer in humans.

3. As part of the Tumor Antigen Project, my laboratory has been analyzing differential expression of various genes in tumor cells relative to normal cells. The purpose of this research is to identify proteins that are abundantly expressed on certain tumor cells and that are either (i) not expressed, or (ii) expressed at lower levels, on corresponding normal cells. We call such differentially expressed proteins "tumor antigen proteins". When such a tumor antigen protein is identified, one can produce an antibody that recognizes and binds to that protein. Such an antibody finds use in the diagnosis of human cancer and may ultimately serve as an effective therapeutic in the treatment of human cancer.

4. In the course of the research conducted by Genentech's Tumor Antigen Project, we have employed a variety of scientific techniques for detecting and studying differential gene expression in human tumor cells relative to normal cells, at genomic DNA, mRNA and protein levels. An important example of one such technique is the well known and widely used technique of microarray analysis which has proven to be extremely useful for the identification of mRNA molecules that are differentially expressed in one tissue or cell type relative to another. In the course of our research using microarray analysis, we have identified approximately 200 gene transcripts that are present in human tumor cells at significantly higher levels than in corresponding normal human cells. To date, we have generated antibodies that bind to about 30 of the tumor antigen proteins expressed from these differentially expressed gene transcripts and have used these antibodies to quantitatively determine the level of production of these tumor antigen proteins in both human cancer cells and corresponding normal cells. We have then compared the levels of mRNA and protein in both the tumor and normal cells analyzed.

5. From the mRNA and protein expression analyses described in paragraph 4 above, we have observed that there is a strong correlation between changes in the level of mRNA present in any particular cell type and the level of protein

expressed from that mRNA in that cell type. In approximately 80% of our observations we have found that increases in the level of a particular mRNA correlates with changes in the level of protein expressed from that mRNA when human tumor cells are compared with their corresponding normal cells.

6. Based upon my own experience accumulated in more than 20 years of research, including the data discussed in paragraphs 4 and 5 above and my knowledge of the relevant scientific literature, it is my considered scientific opinion that for human genes, an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell. In fact, it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein. While there have been published reports of genes for which such a correlation does not exist, it is my opinion that such reports are exceptions to the commonly understood general rule that increased mRNA levels are predictive of corresponding increased levels of the encoded protein.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 5/07/04

By: Paul Polakis

Paul Polakis, Ph.D.

SV 2031808 v1

CURRICULUM VITAE

PAUL G. POLAKIS
Staff Scientist
Genentech, Inc
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S. San Francisco, CA 94080

EDUCATION:

Ph.D., Biochemistry, Department of Biochemistry,
Michigan State University (1984)

B.S., Biology. College of Natural Science, Michigan State University (1977)

PROFESSIONAL EXPERIENCE:

2002-present	Staff Scientist, Genentech, Inc S. San Francisco, CA
1999- 2002	Senior Scientist, Genentech, Inc., S. San Francisco, CA
1997 -1999	Research Director Onyx Pharmaceuticals, Richmond, CA
1992- 1996	Senior Scientist, Project Leader, Onyx Pharmaceuticals, Richmond, CA
1991-1992	Senior Scientist, Chiron Corporation, Emeryville, CA.
1989-1991	Scientist, Cetus Corporation, Emeryville CA.
1987-1989	Postdoctoral Research Associate, Genentech, Inc., South SanFrancisco, CA.
1985-1987	Postdoctoral Research Associate, Department of Medicine, Duke University Medical Center, Durham, NC

1984-1985	Assistant Professor, Department of Chemistry, Oberlin College, Oberlin, Ohio
1980-1984	Graduate Research Assistant, Department of Biochemistry, Michigan State University East Lansing, Michigan

PUBLICATIONS:

1. **Polakis, P G.** and Wilson, J. E. 1982 Purification of a Highly Bindable Rat Brain Hexokinase by High Performance Liquid Chromatography. **Biochem. Biophys. Res. Commun**. 107, 937-943.

2. **Polakis, P.G.** and Wilson, J. E. 1984 Proteolytic Dissection of Rat Brain Hexokinase: Determination of the Cleavage Pattern during Limited Digestion with Trypsin. **Arch. Biochem. Biophys**. 234, 341-352.

3. **Polakis, P. G.** and Wilson, J. E. 1985 An Intact Hydrophobic N-Terminal Sequence is Required for the Binding Rat Brain Hexokinase to Mitochondria. **Arch. Biochem. Biophys**. 236, 328-337.

4. Uhing, R.J., **Polakis,P.G.** and Snyderman, R. 1987 Isolaton of GTP-binding Proteins from Myeloid HL60 Cells. **J. Biol. Chem**. 262, 15575-15579.

5. **Polakis, P.G.**, Uhing, R.J. and Snyderman, R. 1988 The Formylpeptide Chemoattractant Receptor Copurifies with a GTP-binding Protein Containing a Distinct 40 kDa Pertussis Toxin Substrate. **J. Biol. Chem**. 263, 4969-4979.

6. Uhing, R. J., Dillon, S., **Polakis, P. G.**, Truett, A. P. and Snyderman, R. 1988 Chemoattractant Receptors and Signal Transduction Processes in Cellular and Molecular Aspects of Inflammation (Poste, G. and Crooke, S. T. eds.) pp 335-379.

7. **Polakis, P.G.**, Evans, T. and Snyderman 1989 Multiple Chromatographic Forms of the Formylpeptide Chemoattractant Receptor and their Relationship to GTP-binding Proteins. **Biochem. Biophys. Res. Commun**. 161, 276-283.

8. **Polakis, P. G.**, Snyderman, R. and Evans, T. 1989 Characterization of G25K, a GTP-binding Protein Containing a Novel Putative Nucleotide Binding Domain. **Biochem. Biophys. Res. Comun.** 160, 25-32.

9. **Polakis, P.**, Weber,R.F., Nevins,B., Didsbury, J. Evans,T. and Snyderman, R. 1989 Identification of the ral and rac1 Gene Products, Low Molecular Mass GTP-binding Proteins from Human Platelets. **J. Biol. Chem**. 264, 16383-16389.

10. Snyderman, R., Perianin, A., Evans, T., **Polakis, P.** and Didsbury, J. 1989 G Proteins and Neutrophil Function. In ADP-Ribosylating Toxins and G Proteins: Insights into Signal Transduction. (J. Moss and M. Vaughn, eds.) Amer. Soc. Microbiol. pp. 295-323.

11. Hart, M.J., **Polakis, P.G.**, Evans, T. and Cerrione, R.A. 1990 The Identification and Charaterization of an Epidermal Growth Factor-Stimulated Phosphorylation of a Specific Low Molecular Mass GTP-binding Protein in a Reconstituted Phospholipid Vesicle System. **J. Biol. Chem.** 265, 5990-6001.

12. Yatani, A., Okabe, K., **Polakis, P.** Halenbeck, R. McCormick, F. and Brown, A. M. 1990 ras p21 and GAP Inhibit Coupling of Muscarinic Receptors to Atrial K^+ Channels. **Cell**. 61, 769-776.

13. Munemitsu, S., Innis, M.A., Clark, R., McCormick, F., Ullrich, A. and **Polakis, P.G.** 1990 Molecular Cloning and Expression of a G25K cDNA, the Human Homolog of the Yeast Cell Cycle Gene CDC42. **Mol. Cell. Biol.** 10, 5977-5982.

14. Polakis, P.G. Rubinfeld, B. Evans, T. and McCormick, F. 1991 Purification of Plasma Membrane-Associated GTPase Activating Protein (GAP) Specific for rap-1/krev-1 from HL60 Cells. **Proc. Natl. Acad. Sci. USA** 88, 239-243.

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61 Polakis P, Hart M and Rubinfeld B. 1999 Defects in the regulation of beta-catenin
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62 Shen Z, Batzer A, Koehler JA, **Polakis P**, Schlessinger J, Lydon NB, Moran MF. 1999 Evidence for SH3 domain directed binding and phosphorylation of Sam68 by Src. **Oncogene.** 18, 4647-53

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Rastelli, L., **Polakis, P.** and Pennica, D. 2001 Overexpression of the Retinoic Acid-
Responsive Gene Stra6 in Human Cancers and its Synergistic Induction by Wnt-1 and
Retinoic Acid. **Cancer Res** 61, 4197-4204.

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Williams PM, Wieand D, Smith V, Schwall RH, Pennnica D, **Polakis P.** 2002 Synergistic activation of tumor antigens by wnt-1 signaling and retinoic acid revealed by gene expression profiling. **J Biol Chem.** 277,14329-14335.

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75. Shibamoto, S., Winer, J., Williams, M., Polakis, P. 2003 A Blockade in Wnt signaling is activated following the differentiation of F9 teratocarcinoma cells. **Exp. Cell Res.** 29211-20.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Ashkenazi et al.
App. No.	:	09/903,925
Filed	:	July 11, 2001
For	:	SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME
Examiner	:	Hamud, Fozia M

Group Art Unit 1647

CERTIFICATE OF EXPRESS MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to Commissioner of Patents, Washington D.C. 20231 on:

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Commissioner of Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF AVI ASHKENAZI, Ph.D UNDER 37 C.F.R. § 1.132

I, Avi Ashkenazi, Ph.D. declare and say as follows: -

1. I am Director and Staff Scientist at the Molecular Oncology Department of Genentech, Inc., South San Francisco, CA 94080.

2. I joined Genentech in 1988 as a postdoctoral fellow. Since then, I have investigated a variety of cellular signal transduction mechanisms, including apoptosis, and have developed technologies to modulate such mechanisms as a means of therapeutic intervention in cancer and autoimmune disease. I am currently involved in the investigation of a series of secreted proteins over-expressed in tumors, with the aim to identify useful targets for the development of therapeutic antibodies for cancer treatment.

3. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

4. Gene amplification is a process in which chromosomes undergo changes to contain multiple copies of certain genes that normally exist as a single copy, and is an important factor in the pathophysiology of cancer. Amplification of certain genes (e.g., Myc or Her2/Neu)

gives cancer cells a growth or survival advantage relative to normal cells, and might also provide a mechanism of tumor cell resistance to chemotherapy or radiotherapy.

5. If gene amplification results in over-expression of the mRNA and the corresponding gene product, then it identifies that gene product as a promising target for cancer therapy, for example by the therapeutic antibody approach. Even in the absence of over-expression of the gene product, amplification of a cancer marker gene - as detected, for example, by the reverse transcriptase TaqMan® PCR or the fluorescence *in situ* hybridization (FISH) assays -is useful in the diagnosis or classification of cancer, or in predicting or monitoring the efficacy of cancer therapy. An increase in gene copy number can result not only from intrachromosomal changes but also from chromosomal aneuploidy. It is important to understand that detection of gene amplification can be used for cancer diagnosis even if the determination includes measurement of chromosomal aneuploidy. Indeed, as long as a significant difference relative to normal tissue is detected, it is irrelevant if the signal originates from an increase in the number of gene copies per chromosome and/or an abnormal number of chromosomes.

6. I understand that according to the Patent Office, absent data demonstrating that the increased copy number of a gene in certain types of cancer leads to increased expression of its product, gene amplification data are insufficient to provide substantial utility or well established utility for the gene product (the encoded polypeptide), or an antibody specifically binding the encoded polypeptide. However, even when amplification of a cancer marker gene does not result in significant over-expression of the corresponding gene product, this very absence of gene product over-expression still provides significant information for cancer diagnosis and treatment. Thus, if over-expression of the gene product does not parallel gene amplification in certain tumor types but does so in others, then parallel monitoring of gene amplification and gene product over-expression enables more accurate tumor classification and hence better determination of suitable therapy. In addition, absence of over-expression is crucial information for the practicing clinician. If a gene is amplified but the corresponding gene product is not over-expressed, the clinician accordingly will decide not to treat a patient with agents that target that gene product.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so

made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: Avi Ashkenazi Date: 9/15/03

Avi Ashkenazi, Ph.D.

SV 455281 v1
9/12/03 3:06 PM (39780.7000)

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Avi Ashkenazi

July 2003

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Education:

1983:	B.S. in Biochemistry, with honors, Hebrew University, Israel
1986:	Ph.D. in Biochemistry, Hebrew University, Israel

Employment:

1983-1986:	Teaching assistant, undergraduate level course in Biochemistry
1985-1986:	Teaching assistant, graduate level course on Signal Transduction
1986 - 1988:	Postdoctoral fellow, Hormone Research Dept., UCSF, and Developmental Biology Dept., Genentech, Inc., with J. Ramachandran
1988 - 1989:	Postdoctoral fellow, Molecular Biology Dept., Genentech, Inc., with D. Capon
1989 - 1993:	Scientist, Molecular Biology Dept., Genentech, Inc.
1994 -1996:	Senior Scientist, Molecular Oncology Dept., Genentech, Inc.
1996-1997:	Senior Scientist and Interim director, Molecular Oncology Dept., Genentech, Inc.
1997-1990:	Senior Scientist and preclinical project team leader, Genentech, Inc.
1999 -2002:	Staff Scientist in Molecular Oncology, Genentech, Inc.
2002-present:	Staff Scientist and Director in Molecular Oncology, Genentech, Inc.

Awards:

1988:	First prize, The Boehringer Ingelheim Award

Editorial:

Editorial Board Member: Current Biology
Associate Editor, Clinical Cancer Research.
Associate Editor, Cancer Biology and Therapy.

Refereed papers:

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8. Immunoadhesins: Principles and Applications. Gordon Research Conference on Drug Delivery in Biology and Medicine. Ventura, CA, February 1996.

9. Apo-2 Ligand, a new member of the TNF family that induces apoptosis in tumor cells. Cambridge Symposium on TNF and Related Cytokines in Treatment of Cancer. Hilton-Head, NC, March 1996.
10. Induction of apoptosis by Apo2 Ligand. American Society for Biochemistry and Molecular Biology, Symposium on Growth Factors and Cytokine Receptors. New Orleans, LA, June, 1996.
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16. Control of Apo2L signaling. Cold Spring Harbor Laboratory Symposium on Programmed Cell Death. Cold Spring Harbor, New York. September, 1997.
17. Chairman and speaker, Apoptosis Signaling session. IBC's 4th Annual Conference on Apoptosis. San Diego, CA., October 1997.
18. Control of Apo2L signaling by death and decoy receptors. American Association for the Advancement of Science. Philladelphia, PA, February 1998.
19. Apo2 ligand and its receptors. American Society of Immunologists. San Francisco, CA, April 1998.
20. Death receptors and ligands. 7th International TNF Congress. Cape Cod, MA, May 1998.
21. Apo2L as a potential therapeutic for cancer. UCLA School of Medicine. LA, CA, June 1998.
22. Apo2L as a potential therapeutic for cancer. Gordon Research Conference on Cancer Chemotherapy. New London, NH, July 1998.
23. Control of apoptosis by Apo2L. Endocrine Society Conference, Stevenson, WA, August 1998.
24. Control of apoptosis by Apo2L. International Cytokine Society Conference, Jerusalem, Israel, October 1998.

25. Apoptosis control by death and decoy receptors. American Association for Cancer Research Conference, Whistler, BC, Canada, March 1999.
26. Apoptosis control by death and decoy receptors. American Society for Biochemistry and Molecular Biology Conference, San Francisco, CA, May 1999.
27. Apoptosis control by death and decoy receptors. Gordon Research Conference on Apoptosis, New London, NH, June 1999.
28. Apoptosis control by death and decoy receptors. Arthritis Foundation Research Conference, Alexandria GA, Aug 1999.
29. Safety and anti-tumor activity of recombinant soluble Apo2L/TRAIL. Cold Spring Harbor Laboratory Symposium on Programmed Cell Death. . Cold Spring Harbor, NY, September 1999.
30. The Apo2L/TRAIL system: therapeutic potential. American Association for Cancer Research, Lake Tahoe, NV, Feb 2000.
31. Apoptosis and cancer therapy. Stanford University School of Medicine, Stanford, CA, Mar 2000.
32. Apoptosis and cancer therapy. University of Pennsylvania School of Medicine, Philladelphia, PA, Apr 2000.
33. Apoptosis signaling by Apo2L/TRAIL. International Congress on TNF. Trondheim, Norway, May 2000.
34. The Apo2L/TRAIL system: therapeutic potential. Cap-CURE summit meeting. Santa Monica, CA, June 2000.
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44. Targeting death receptors in cancer with Apo2L/TRAIL. Cell Death and Disease conference, North Falmouth, MA, Jun 2001.
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46. Apo2L/TRAIL signaling and apoptosis resistance mechanisms. Gordon Research Conference on Apoptosis, Oxford, UK, July 2001.
47. Apo2L/TRAIL signaling and apoptosis resistance mechanisms. Cleveland Clinic Foundation, Cleveland, OH, Oct 2001.
48. Apoptosis signaling by death receptors: overview. International Society for Interferon and Cytokine Research conference, Cleveland, OH, Oct 2001.
49. Apoptosis signaling by death receptors. American Society of Nephrology Conference. San Francisco, CA, Oct 2001.
50. Targeting death receptors in cancer. Apoptosis: commercial opportunities. San Diego, CA, Apr 2002.
51. Apo2L/TRAIL signaling and apoptosis resistance mechanisms. Kimmel Cancer Research Center, Johns Hopkins University, Baltimore MD. May 2002.
52. Apoptosis control by Apo2L/TRAIL. (Keynote Address) University of Alabama Cancer Center Retreat, Birmingham, Ab. October 2002.
53. Apoptosis signaling by Apo2L/TRAIL. (Session co-chair) TNF international conference. San Diego, CA. October 2002.
54. Apoptosis signaling by Apo2L/TRAIL. Swiss Institute for Cancer Research (ISREC). Lausanne, Swizerland. Jan 2003.
55. Apoptosis induction with Apo2L/TRAIL. Conference on New Targets and Innovative Strategies in Cancer Treatment. Monte Carlo. February 2003.
56. Apoptosis signaling by Apo2L/TRAIL. Hermelin Brain Tumor Center Symposium on Apoptosis. Detroit, MI. April 2003.
57. Targeting apoptosis through death receptors. Sixth Annual Conference on Targeted Therapies in the Treatment of Breast Cancer. Kona, Hawaii. July 2003.
58. Targeting apoptosis through death receptors. Second International Conference on Targeted Cancer Therapy. Washington, DC. Aug 2003.

Issued Patents:

1. Ashkenazi, A., Chamow, S. and Kogan, T. Carbohydrate-directed crosslinking reagents. US patent 5,329,028 (Jul 12, 1994).
2. Ashkenazi, A., Chamow, S. and Kogan, T. Carbohydrate-directed crosslinking reagents. US patent 5,605,791 (Feb 25, 1997).
3. Ashkenazi, A., Chamow, S. and Kogan, T. Carbohydrate-directed crosslinking reagents. US patent 5,889,155 (Jul 27, 1999).
4. Ashkenazi, A., APO-2 Ligand. US patent 6,030,945 (Feb 29, 2000).
5. Ashkenazi, A., Chuntharapai, A., Kim, J., APO-2 ligand antibodies. US patent 6, 046, 048 (Apr 4, 2000).
6. Ashkenazi, A., Chamow, S. and Kogan, T. Carbohydrate-directed crosslinking reagents. US patent 6,124,435 (Sep 26, 2000).
7. Ashkenazi, A., Chuntharapai, A., Kim, J., Method for making monoclonal and cross-reactive antibodies. US patent 6,252,050 (Jun 26, 2001).
8. Ashkenazi, A. APO-2 Receptor. US patent 6,342,369 (Jan 29, 2002).
9. Ashkenazi, A. Fong, S., Goddard, A., Gurney, A., Napier, M., Tumas, D., Wood, W. A-33 polypeptides. US patent 6,410,708 (Jun 25, 2002).
10. Ashkenazi, A. APO-3 Receptor. US patent 6,462,176 B1 (Oct 8, 2002).
11. Ashkenazi, A. APO-2LI and APO-3 polypeptide antibodies. US patent 6,469,144 B1 (Oct 22, 2002).
12. Ashkenazi, A., Chamow, S. and Kogan, T. Carbohydrate-directed crosslinking reagents. US patent 6,582,928B1 (Jun 24, 2003).

GenBank (Release 142, jun 2004)

494 100 0.0
P_AAF92072 Human PRO831 cDNA. 494 bp, cDNA, PAT 15-MAY-2001
ACCESSION P_AAF92072
KEYWORDS GENESEQ; Human; PRO protein; mapping; patent; patentdb (v200414, 01-JUL-2004).
SOURCE Homo sapiens.
ORGANISM Homo sapiens.
REFERENCE 1 (bases 1 to 494)
AUTHORS Eaton,D.L., Filvaroff,E., Gerritsen,M.E., Goddard,A., Godowski,P.J. Grimaldi,C.J., Gurney,A.L., Watanabe,C.K., Wood,W.I.
TITLE Eighty four nucleic acids encoding PRO polypeptides, useful in molecular biology, including use as hybridization probes, and in chromosome and gene mapping.
JOURNAL Patent: WO200116318-A2; Filing Date: 24-AUG-2000; 2000WO-US023328; Publication Date: 08-MAR-2001; Priority: 01-SEP-1999; 99WO-US020111. 15-SEP-1999; 99WO-US021090. 07-DEC-1999; 99US-0169495P. 09-DEC-1999; 99US-0170262P. 11-JAN-2000; 2000US-0175481P. 18-FEB-2000; 2000WO-US004341. 18-FEB-2000; 2000WO-US004342. 22-FEB-2000; 2000WO-US004414. 01-MAR-2000; 2000WO-US005601. 03-MAR-2000; 2000US-0187202P. 21-MAR-2000; 2000US-0191007P. 30-MAR-2000; 2000WO-US008439. 25-APR-2000; 2000US-0199397P. 22-MAY-2000; 2000WO-US014042. 05-JUN-2000; 2000US-0209832P; Assignee: (GETH) GENENTECH INC; Cross Reference: WPI; 2001-183260/18. P-PSDB; AAB87540; Patent Format: Claim 2; Fig 29; 278pp; English.
COMMENT The present sequence is the coding sequence for a human PRO polypeptide (secreted and transmembrane). The PRO protein, and PRO agonists, PRO antagonists or anti-PRO antibodies are useful for preparation of a medicament useful in the treatment of a condition which is responsive to the PRO protein, agonists, antagonists or anti-PRO antibodies. The PRO protein may also be employed as molecular weight markers for protein electrophoresis. The PRO coding sequence has applications in molecular biology, including use as hybridisation probes, and in chromosome and gene mapping
FEATURES Location/Qualifiers
BASE COUNT 128 a 111 c 120 g 135 t
ORIGIN

494 100 0.0
AX092298 Sequence 29 from Patent WO0116318. 494 bp, DNA, linear, PAT 21-MAR-2001
ACCESSION AX092298
VERSION AX092298.1 GI:13444463
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1
AUTHORS Eaton,D.L., Filvaroff,E., Gerritsen,M.E., Goddard,A., Godowski,P.J., Grimaldi,C.J., Gurney,A.L., Watanabe,C.K. and Wood,W.I.
TITLE Secreted and transmembrane polypeptides and nucleic acids encoding the same

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  JOURNAL    Patent: WO 0116318-A 29 08-MAR-2001;
           Genentech, Inc. (US)
FEATURES              Location/Qualifiers
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BASE COUNT
ORIGIN

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P_AAA37028  Human PRO831 (UNQ471) cDNA sequence SEQ ID NO:21. 493 bp,
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ACCESSION    P_AAA37028
KEYWORDS     GENESEQ; Human; PRO polypeptide; membrane bound protein; receptor;
           diagnosis; transmembrane; secretion; immunoadhesion; pharmaceutical;
           screening;  patent; patentdb (v200414, 01-JUL-2004).
SOURCE       Homo sapiens.
  ORGANISM   Homo sapiens.
REFERENCE    1  (bases 1 to 493)
  AUTHORS    Baker,K.,  Goddard,A.,  Gurney,A.L.,  Smith,V.,  Watanabe,C.K.,
           Wood,W.I.
  TITLE      New mammalian DNA sequences encoding transmembrane, receptor or
           secreted  PRO polypeptides, useful for screening of potential
           peptide or small  molecule inhibitors of the relevant
           receptor/ligand interactions.
  JOURNAL    Patent: WO200012708-A2; Filing Date: 01-SEP-1999;  99WO-US020111;
           Publication Date: 09-MAR-2000; Priority: 01-SEP-1998;
           98US-0098716P. 01-SEP-1998;   98US-0098749P. 01-SEP-1998;
           98US-0098750P. 02-SEP-1998;   98US-0098803P. 02-SEP-1998;
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           98US-0099642P. 10-SEP-1998;   98US-0099741P. 10-SEP-1998;
           98US-0099754P. 10-SEP-1998;   98US-0099763P. 10-SEP-1998;
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           98US-0101477P. 23-SEP-1998;   98US-0101479P. 24-SEP-1998;
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           98US-0101743P. 24-SEP-1998;   98US-0101915P. 24-SEP-1998;
           98US-0101916P. 29-SEP-1998;   98US-0102207P. 29-SEP-1998;
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98US-0102570P. 30-SEP-1998; 98US-0102571P. 01-OCT-1998;
98US-0102684P. 01-OCT-1998; 98US-0102687P. 02-OCT-1998;
98US-0102965P. 06-OCT-1998; 98US-0103258P. 06-OCT-1998;
98US-0103449P. 07-OCT-1998; 98US-0103314P. 07-OCT-1998;
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98US-0103395P. 07-OCT-1998; 98US-0103396P. 07-OCT-1998;
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98US-0103711P. 14-OCT-1998; 98US-0104257P. 20-OCT-1998;
98US-0104987P. 20-OCT-1998; 98US-0105000P. 20-OCT-1998;
98US-0105002P. 21-OCT-1998; 98US-0105104P. 22-OCT-1998;
98US-0105169P. 22-OCT-1998; 98US-0105266P. 26-OCT-1998;
98US-0105693P. 26-OCT-1998; 98US-0105694P. 27-OCT-1998;
98US-0105807P. 27-OCT-1998; 98US-0105881P. 27-OCT-1998;
98US-0105882P. 27-OCT-1998; 98US-0106062P. 28-OCT-1998;
98US-0106023P. 28-OCT-1998; 98US-0106029P. 28-OCT-1998;
98US-0106030P. 28-OCT-1998; 98US-0106032P. 28-OCT-1998;
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98US-0106248P. 29-OCT-1998; 98US-0106384P. 29-OCT-1998;
98US-0108500P. 30-OCT-1998; 98US-0106464P. 03-NOV-1998;
98US-0106856P. 03-NOV-1998; 98US-0106902P. 03-NOV-1998;
98US-0106905P. 03-NOV-1998; 98US-0106919P. 03-NOV-1998;
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98US-0107783P. 17-NOV-1998; 98US-0108775P. 17-NOV-1998;
98US-0108779P. 17-NOV-1998; 98US-0108787P. 17-NOV-1998;
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98US-0108802P. 17-NOV-1998; 98US-0108806P. 17-NOV-1998;
98US-0108807P. 17-NOV-1998; 98US-0108867P. 17-NOV-1998;
98US-0108925P. 18-NOV-1998; 98US-0108848P. 18-NOV-1998;
98US-0108849P. 18-NOV-1998; 98US-0108850P. 18-NOV-1998;
98US-0108851P. 18-NOV-1998; 98US-0108852P. 18-NOV-1998;
98US-0108858P. 18-NOV-1998; 98US-0108904P; Assignee: (GETH)
GENENTECH INC; Cross Reference: WPI; 2000-237871/20. P-PSDB;
AAY99346; Patent Format: Claim 2; Fig 13; 773pp; English.

COMMENT AAA37022 to AAA37144 encode the new isolated human transmembrane, receptor or secreted PRO polypeptides given in AAY99340 to AAY99462. The transmembrane and receptor PRO proteins can be used for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interactions. The polypeptides and nucleotide sequences encoding then have various industrial applications, including uses as pharmaceutical and diagnostic agents. AAA37145 to AAA37330 represent PCR primers and hybridisation probes used in the isolation of the PRO polypeptides from the present invention

FEATURES Location/Qualifiers
BASE COUNT 127 a 111 c 120 g 135 t
ORIGIN

486 100 0.0
BC021104 Homo sapiens apelin, AGTRL1 ligand, mRNA (cDNA clone MGC:31846 IMAGE:4586949), complete cds. 2673 bp, mRNA, linear, PRI 30-JUN-2004
ACCESSION BC021104
VERSION BC021104.1 GI:18088893
KEYWORDS MGC.
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens

Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.

REFERENCE 1 (bases 1 to 2673)

AUTHORS Strausberg,R.L., Feingold,E.A., Grouse,L.H., Derge,J.G., Klausner,R.D., Collins,F.S., Wagner,L., Shenmen,C.M., Schuler,G.D., Altschul,S.F., Zeeberg,B., Buetow,K.H., Schaefer,C.F., Bhat,N.K., Hopkins,R.F., Jordan,H., Moore,T., Max,S.I., Wang,J., Hsieh,F., Diatchenko,L., Marusina,K., Farmer,A.A., Rubin,G.M., Hong,L., Stapleton,M., Soares,M.B., Bonaldo,M.F., Casavant,T.L., Scheetz,T.E., Brownstein,M.J., Usdin,T.B., Toshiyuki,S., Carninci,P., Prange,C., Raha,S.S., Loquellano,N.A., Peters,G.J., Abramson,R.D., Mullahy,S.J., Bosak,S.A., McEwan,P.J., McKernan,K.J., Malek,J.A., Gunaratne,P.H., Richards,S., Worley,K.C., Hale,S., Garcia,A.M., Gay,L.J., Hulyk,S.W., Villalon,D.K., Muzny,D.M., Sodergren,E.J., Lu,X., Gibbs,R.A., Fahey,J., Helton,E., Ketteman,M., Madan,A., Rodrigues,S., Sanchez,A., Whiting,M., Madan,A., Young,A.C., Shevchenko,Y., Bouffard,G.G., Blakesley,R.W., Touchman,J.W., Green,E.D., Dickson,M.C., Rodriguez,A.C., Grimwood,J., Schmutz,J., Myers,R.M., Butterfield,Y.S., Krzywinski,M.I., Skalska,U., Smailus,D.E., Schnerch,A., Schein,J.E., Jones,S.J. and Marra,M.A.

TITLE Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences

JOURNAL Proc. Natl. Acad. Sci. U.S.A. 99 (26), 16899-16903 (2002)

REFERENCE 2 (bases 1 to 2673)

AUTHORS Strausberg,R.

TITLE Direct Submission

JOURNAL Submitted (03-JAN-2002) National Institutes of Health, Mammalian Gene Collection (MGC), Cancer Genomics Office, National Cancer Institute, 31 Center Drive, Room 11A03, Bethesda, MD 20892-2590, USA

REMARK NIH-MGC Project URL: http://mgc.nci.nih.gov

COMMENT Contact: MGC help desk
Email: cgapbs-r@mail.nih.gov
Tissue Procurement: DCTD/DTP
cDNA Library Preparation: Rubin Laboratory
cDNA Library Arrayed by: The I.M.A.G.E. Consortium (LLNL)
DNA Sequencing by: Genome Sequence Centre,
BC Cancer Agency, Vancouver, BC, Canada
info@bcgsc.bc.ca
Steve Jones, Sarah Barber, Mabel Brown-John, Yaron Butterfield, Andy Chan, Steve S. Chand, William Chow, Alison Cloutier, Ruth Featherstone, Malachi Griffith, Obi Griffith, Ran Guin, Nancy Liao, Kim MacDonald, Amara Masson, Mike R. Mayo, Josh Moran, Ryan Morin, Teika Olson, Diana Palmquist, Anca Petrescu, Anna Liisa Prahbu, Parvaneh Saeedi, JR Santos, Angelique Schnerch, Ursula Skalska, Duane Smailus, Jeff Stott, Miranda Tsai, George Yang, Jacquie Schein, Asim Siddiqui, Rob Holt, Marco Marra.

Clone distribution: MGC clone distribution information can be found through the I.M.A.G.E. Consortium/LLNL at: http://image.llnl.gov
Series: IRAL Plate: 40 Row: f Column: 18
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BASE COUNT
ORIGIN

468 100   0.0
P_AAX235184Human kidney aminopeptidase P genomic DNA fragment 2. 998 bp,
          DNA, PAT 23-JUN-1999
ACCESSION   P_AAX23518
KEYWORDS    GENESEQ; Aminopeptidase; human; AmP; gene therapy; treatment;
          AmP-deficiency; prenatal diagnosis; angioedema; antihypertensive
          agent; atherosclerosis; arterial stenosis; industrial protein feed;
          malabsorption syndrome; proteinaceous waste degradation; additive;
          immunohistochemistry;  patent; patentdb (v200414, 01-JUL-2004).
SOURCE      Homo sapiens.
  ORGANISM  Homo sapiens.
REFERENCE   1  (bases 1 to 49998)
  AUTHORS   Ryan,J.W.,  Sprinkle,T.J.C.,  Venema,R.C.
  TITLE     Nucleic acid encoding human aminopeptidase P.
  JOURNAL   Patent: WO9911799-A2; Filing Date: 02-SEP-1998;   98WO-US018426;
          Publication Date: 11-MAR-1999; Priority: 02-SEP-1997;
          97US-0057854P; Assignee: (MEDI-) MEDICAL COLLEGE GEORGIA RES INST;
          Cross Reference: WPI; 1999-205193/17; Patent Format: Claim 13; Page
          109-139; 201pp; English.
COMMENT     This invention describes the isolation of a novel human
          aminopeptidase P  (AmP). This protein is used to produce recombinant
          AmP and can be used  for gene therapy for treating AmP-deficiency
          conditions. Its fragments  are used as primers and probes to
          identify patients with homozygous and  heterozygous AmP deficiency,
          including prenatal diagnosis (patients  defective in AmP are at risk
          of developing angioedema if treated with  angiotensin-converting
          enzyme inhibitors), also as antisense inhibitors  in cases of
          excessive AmP expression. The product of the invention is  also used
          to identify AmP-expressing sequences in other animals and to
          generate transgenic animals, and comparisons of genomic sequences
          are  used to detect mutations. AmP inhibitors are potentially useful
          as  antihypertensive agents and to prevent or treat arterial
          (re)stenosis or  atherosclerosis. The structure of AmP is used to
          design synthetic  substrates, e.g. for use in AmP assays. AmP, which
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hydrolyzes N-terminal imido bonds, can be used to degrade industrial protein feeds to free amino acids, to degrade proteinaceous wastes, as additives in enzyme formulations used to treat malabsorption syndrome and for studying its biological role. Antibodies against AmP are used in immunohistochemical methods to study AmP distribution

FEATURES Location/Qualifiers
BASE COUNT 12605 a 11725 c 11351 g 14317 t
ORIGIN

468 100 0.0
HS454M7 Human DNA sequence from clone RP3-454M7 on chromosome Xq25-26.3, complete sequence. 151152 bp, DNA, linear, PRI 05-JUN-2003
ACCESSION AL022162
VERSION AL022162.1 GI:3171881
KEYWORDS HTG.
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (bases 1 to 151152)
AUTHORS Pavitt,R.
TITLE Direct Submission
JOURNAL Submitted (05-JUN-2003) Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire, CB10 1SA, UK. E-mail enquiries: humquery@sanger.ac.uk Clone requests: clonerequest@sanger.ac.uk
COMMENT On Jun 2, 1998 this sequence version replaced gi:2969945.
---------- Genome Center
Center: Wellcome Trust Sanger Institute
Center code: SC
Web site: http://www.sanger.ac.uk
Contact: humquery@sanger.ac.uk

During sequence assembly data is compared from overlapping clones. Where differences are found these are annotated as variations together with a note of the overlapping clone name. Note that the variation annotation may not be found in the sequence submission corresponding to the overlapping clone, as we submit sequences with only a small overlap as described above.
This sequence was finished as follows unless otherwise noted: all regions were either double-stranded or sequenced with an alternate chemistry or covered by high quality data (i.e., phred quality }= 30); an attempt was made to resolve all sequencing problems, such as compressions and repeats; all regions were covered by at least one plasmid subclone or more than one M13 subclone; and the assembly was confirmed by restriction digest, except on the rare occasion of the clone being a YAC.
The following abbreviations are used to associate primary accession numbers given in the feature table with their source databases: Em:, EMBL; Sw:, SWISSPROT; Tr:, TREMBL; Wp:, WORMPEP; Information on the WORMPEP database can be found at http://www.sanger.ac.uk/Projects/C_elegans/wormpep This sequence was generated from part of bacterial clone contigs of human chromosome X, constructed by the Sanger Centre Chromosome X Mapping Group. Further information can be found at http://www.sanger.ac.uk/HGP/ChrX
RP3-454M7 is from the library RPCI-3 constructed by the group of

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          Pieter de Jong. For further details see
          http://www.chori.org/bacpac/home.htm
          VECTOR: pCYPAC2
          This sequence is the entire insert of clone RP3-454M7.
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                     /note="variant 1
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     CDS             join(<769..877,1303..1392,2075..2195,2282..2443,
                     3992..4093,4621..4735,5826..5942,6041..6228,9214..9325,
                     10696..10805,12706..12841,18582..18692,19339..19504,
                     19759..19994,27786..27809,30444..30560,31621..31705,
                     32328..32455,33287..33398,33588..33712)
                     /gene="OCRL1"
                     /codon_start=1
                     /evidence=not_experimental
                     /product="dJ454M7.1.2 (variant 2)"
                     /protein_id="CAA18150.1"
                     /db_xref="GI:3171882"
     CDS             join(<769..877,1303..1392,2075..2195,2282..2443,
                     3992..4093,4621..4735,5826..5942,6041..6228,9214..9325,
                     10696..10805,12706..12841,18582..18692,19339..19504,
                     19759..19994,30444..30560,31621..31705,32328..32455,
                     33287..33398,33588..33712)
                     /gene="OCRL1"
                     /note="variant 1"
                     /codon_start=1
```

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                     /evidence=not_experimental
                     /product="dJ454M7.1.1 (Lowe Oculocerebrorenal Syndrome)"
                     /protein_id="CAA18151.1"
                     /db_xref="GI:4160528"
     repeat_region   1586..1847
                     /note="MIR repeat: matches 2..262 of consensus"
     repeat_region   2642..2940
                     /note="AluJb repeat: matches 1..302 of consensus"
     repeat_region   6639..6933
                     /note="MER33 repeat: matches 1..323 of consensus"
     repeat_region   7178..8315
                     /note="L1PA2 repeat: matches 5000..6146 of consensus"
     repeat_region   11225..11294
                     /note="MER5B repeat: matches 109..178 of consensus"
     repeat_region   11345..11657
                     /note="AluYb8 repeat: matches 1..310 of consensus"
     repeat_region   11736..11797
                     /note="31 copies 2 mer tt 72% conserved"
     repeat_region   12436..12562
                     /note="L2 repeat: matches 2579..2710 of consensus"
     repeat_region   13276..13457
                     /note="AluSg/x repeat: matches 129..310 of consensus"
     repeat_region   13679..13873
                     /note="MIR repeat: matches 5..213 of consensus"
     repeat_region   14132..14206
                     /note="MIR repeat: matches 163..233 of consensus"
     repeat_region   14207..14413
                     /note="MER8 repeat: matches 2..239 of consensus"
     repeat_region   14414..14548
                     /note="MIR repeat: matches 12..163 of consensus"
     repeat_region   14793..15073
                     /note="AluY repeat: matches 1..311 of consensus"
     repeat_region   15383..15589
                     /note="MER3 repeat: matches 1..209 of consensus"
     repeat_region   16139..16279
                     /note="MER5B repeat: matches 29..178 of consensus"
     repeat_region   16317..16466
                     /note="MIR repeat: matches 52..212 of consensus"
     repeat_region   16667..17000
                     /note="L1PA9 repeat: matches 5829..6163 of consensus"
     repeat_region   17001..17301
                     /note="AluYb8 repeat: matches 1..302 of consensus"
     repeat_region   17302..17642
                     /note="L1PA9 repeat: matches 5491..5829 of consensus"
     repeat_region   18285..18620
                     /note="2 copies 168 mer 78% conserved"
     repeat_region   18772..18981
                     /note="MIR repeat: matches 6..242 of consensus"
     repeat_region   18945..19005
                     /note="L2 repeat: matches 2648..2702 of consensus"
     repeat_region   20349..20489
                     /note="MIR repeat: matches 1..150 of consensus"
     repeat_region   20543..20697
                     /note="MER5A repeat: matches 22..188 of consensus"
     repeat_region   21496..21830
                     /note="L1MC4 repeat: matches 7477..7849 of consensus"
     repeat_region   22420..22650
```

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                /note="MER46A repeat: matches 1..236 of consensus"
repeat_region   22894..22965
                /note="3 copies 24 mer 83% conserved"
repeat_region   24309..24606
                /note="AluSx repeat: matches 1..299 of consensus"
repeat_region   25081..25134
                /note="27 copies 2 mer ta 70% conserved"
repeat_region   25168..25193
                /note="13 copies 2 mer tg 100% conserved"
repeat_region   25262..25301
                /note="20 copies 2 mer tc 90% conserved"
repeat_region   25302..25642
                /note="L2 repeat: matches 2078..2419 of consensus"
repeat_region   26542..26704
                /note="MIR repeat: matches 1..160 of consensus"
repeat_region   26822..27057
                /note="MIR repeat: matches 8..255 of consensus"
repeat_region   28330..28399
                /note="MIR repeat: matches 82..151 of consensus"
repeat_region   30715..30819
                /note="MIR repeat: matches 35..138 of consensus"
repeat_region   31196..31319
                /note="AluJb repeat: matches 2..125 of consensus"
repeat_region   37202..37675
                /note="L2 repeat: matches 1597..2041 of consensus"
repeat_region   37676..37990
                /note="AluJb repeat: matches 1..312 of consensus"
repeat_region   37991..38142
                /note="L2 repeat: matches 2041..2182 of consensus"
repeat_region   38143..38316
                /note="MER5A repeat: matches 3..189 of consensus"
repeat_region   38317..38464
                /note="L2 repeat: matches 2182..2419 of consensus"
repeat_region   38676..38801
                /note="MIR repeat: matches 11..134 of consensus"
repeat_region   38858..38984
                /note="MIR repeat: matches 123..257 of consensus"
repeat_region   39284..39886
                /note="L2 repeat: matches 2147..2748 of consensus"
repeat_region   39913..40014
                /note="MIR repeat: matches 175..262 of consensus"
repeat_region   40015..40311
                /note="AluSg repeat: matches 1..297 of consensus"
repeat_region   40312..40428
                /note="MIR repeat: matches 14..175 of consensus"
repeat_region   40667..40774
                /note="L2 repeat: matches 2616..2702 of consensus"
repeat_region   40776..41088
                /note="AluSx repeat: matches 1..305 of consensus"
repeat_region   41528..41936
                /note="L2 repeat: matches 2267..2709 of consensus"
repeat_region   42180..42713
                /note="L1MB3 repeat: matches 5584..6153 of consensus"
repeat_region   42714..43014
                /note="AluJb repeat: matches 1..312 of consensus"
repeat_region   43015..43042
                /note="L1MB3 repeat: matches 6153..6178 of consensus"
```

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repeat_region   43197..43238
                /note="5S repeat: matches 1..42 of consensus"
repeat_region   43282..43574
                /note="L2 repeat: matches 2405..2677 of consensus"
repeat_region   45284..45741
                /note="MLT1C repeat: matches 1..466 of consensus"
repeat_region   45835..45905
                /note="L2 repeat: matches 1139..1203 of consensus"
repeat_region   45906..46207
                /note="AluSq repeat: matches 12..313 of consensus"
repeat_region   46208..46584
                /note="L2 repeat: matches 1203..1621 of consensus"
repeat_region   46714..47015
                /note="AluSg repeat: matches 1..302 of consensus"
repeat_region   47016..47159
                /note="MER58B repeat: matches 196..341 of consensus"
repeat_region   47168..47374
                /note="MER30 repeat: matches 1..230 of consensus"
repeat_region   47427..47581
                /note="MER61A repeat: matches 4..158 of consensus"
repeat_region   47611..47774
                /note="AluSx repeat: matches 12..175 of consensus"
repeat_region   48682..48987
                /note="AluY repeat: matches 1..306 of consensus"
repeat_region   49541..49678
                /note="L2 repeat: matches 1906..2046 of consensus"
repeat_region   49698..49898
                /note="MER53 repeat: matches 1..188 of consensus"
repeat_region   49909..49978
                /note="LTR19B repeat: matches 59..140 of consensus"
repeat_region   50220..50423
                /note="L2 repeat: matches 2518..2730 of consensus"
repeat_region   50421..50492
                /note="3 copies 24 mer 93% conserved"
repeat_region   50846..50915
                /note="MIR repeat: matches 48..117 of consensus"
repeat_region   50914..51068
                /note="MIR repeat: matches 34..211 of consensus"
repeat_region   51124..51292
                /note="AluSg/x repeat: matches 130..300 of consensus"
repeat_region   51517..51587
                /note="MER96 repeat: matches 105..175 of consensus"
repeat_region   52716..52853
                /note="MIR repeat: matches 68..213 of consensus"
repeat_region   52994..53224
                /note="MIR repeat: matches 2..258 of consensus"
repeat_region   54036..54154
                /note="MLT1I repeat: matches 292..410 of consensus"
repeat_region   54234..54586
                /note="MLT1A2 repeat: matches 1..373 of consensus"
repeat_region   54612..54704
                /note="MLT1J repeat: matches 106..200 of consensus"
repeat_region   54708..54755
                /note="L2 repeat: matches 2691..2738 of consensus"
repeat_region   55103..55409
                /note="AluYb8 repeat: matches 1..314 of consensus"
repeat_region   55876..56326
```

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                     /note="L2 repeat: matches 2225..2658 of consensus"
     repeat_region   56404..56697
                     /note="AluSg repeat: matches 1..292 of consensus"
     repeat_region   56760..56882
                     /note="L2 repeat: matches 2605..2730 of consensus"
     repeat_region   57036..57226
                     /note="MIR repeat: matches 73..262 of consensus"
     repeat_region   57448..57579
                     /note="L1PA16 repeat: matches 6025..6157 of consensus"
     repeat_region   57817..58128
                     /note="L2 repeat: matches 2434..2746 of consensus"
     repeat_region   58772..59030
                     /note="MIR repeat: matches 1..262 of consensus"
     repeat_region   59040..59091
                     /note="MIR repeat: matches 206..257 of consensus"
     repeat_region   59050..59118
                     /note="L2 repeat: matches 2626..2729 of consensus"
     repeat_region   59465..59797
                     /note="AluSx repeat: matches 1..301 of consensus"
     repeat_region   61554..61833
                     /note="AluSx repeat: matches 6..286 of consensus"
     repeat_region   62011..62239
                     /note="MIR repeat: matches 2..242 of consensus"
     repeat_region   62265..62379
                     /note="L2 repeat: matches 2430..2545 of consensus"
     repeat_region   63472..63529
                     /note="MIR repeat: matches 185..242 of consensus"
     repeat_region   64262..64313
                     /note="MIR repeat: matches 92..143 of consensus"
     repeat_region   64330..64409
                     /note="MER33 repeat: matches 241..324 of consensus"
     repeat_region   64459..64631
                     /note="L1MA10 repeat: matches 5999..6322 of consensus"
     repeat_region   64637..64862
                     /note="MER33 repeat: matches 5..225 of consensus"
     repeat_region   65788..66082
                     /note="AluJo repeat: matches 6..304 of consensus"
     repeat_region   66968..66991
                     /note="L2 repeat: matches 2470..2493 of consensus"
     repeat_region   67036..67653
                     /note="L2 repeat: matches 1882..2511 of consensus"
     repeat_region   67654..67700
                     /note="L1PA16 repeat: matches 4148..4193 of consensus"
     repeat_region   67701..67998
                     /note="AluY repeat: matches 1..296 of consensus"
     repeat_region   67999..68463
                     /note="L1PA16 repeat: matches 4193..4655 of consensus"
     repeat_region   68464..68826
                     /note="THE1B repeat: matches 1..364 of consensus"
     repeat_region   68827..70335
                     /note="L1PA16 repeat: matches 4655..6143 of consensus"
     repeat_region   70349..70538
                     /note="L2 repeat: matches 1697..1895 of consensus"
     repeat_region   70565..70865
                     /note="AluY repeat: matches 1..305 of consensus"
     repeat_region   70932..71126
                     /note="L1ME3 repeat: matches 5734..5939 of consensus"
```

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repeat_region   71385..71690
                /note="AluYa5 repeat: matches 1..310 of consensus"
repeat_region   71717..72253
                /note="MLT1F repeat: matches 11..513 of consensus"
repeat_region   72615..72631
                /note="MIR repeat: matches 196..212 of consensus"
repeat_region   72632..72870
                /note="MER8 repeat: matches 1..239 of consensus"
repeat_region   72871..73028
                /note="MIR repeat: matches 45..196 of consensus"
repeat_region   73892..73979
                /note="MIR repeat: matches 59..147 of consensus"
repeat_region   74431..74484
                /note="27 copies 2 mer ca 96% conserved"
repeat_region   74436..74483
                /note="2 copies 24 mer 100% conserved"
repeat_region   74504..74671
                /note="MIR repeat: matches 2..171 of consensus"
repeat_region   75019..75185
                /note="L2 repeat: matches 2517..2704 of consensus"
repeat_region   75149..75249
                /note="MIR repeat: matches 28..137 of consensus"
repeat_region   75397..75588
                /note="MLT1A1 repeat: matches 1..194 of consensus"
repeat_region   75646..75831
                /note="MLT1A1 repeat: matches 171..365 of consensus"
repeat_region   76554..76826
                /note="AluSg1 repeat: matches 1..308 of consensus"
repeat_region   76863..76903
                /note="L2 repeat: matches 2652..2693 of consensus"
repeat_region   77089..77189
                /note="MIR repeat: matches 41..144 of consensus"
repeat_region   77359..77679
                /note="AluSx repeat: matches 1..301 of consensus"
repeat_region   78584..78722
                /note="L2 repeat: matches 2615..2750 of consensus"
repeat_region   79058..79304
                /note="MIR repeat: matches 8..262 of consensus"
repeat_region   81458..81515
                /note="MIR repeat: matches 95..154 of consensus"
repeat_region   81526..81665
                /note="L2 repeat: matches 2352..2503 of consensus"
repeat_region   81793..81843
                /note="L2 repeat: matches 2706..2750 of consensus"
repeat_region   82019..82082
                /note="MIR repeat: matches 90..153 of consensus"
repeat_region   82664..82739
                /note="L2 repeat: matches 2647..2722 of consensus"
repeat_region   82740..83045
                /note="AluSx repeat: matches 1..312 of consensus"
repeat_region   83046..83071
                /note="L2 repeat: matches 2722..2747 of consensus"
repeat_region   83813..83946
                /note="MIR repeat: matches 89..250 of consensus"
repeat_region   83904..83954
                /note="L2 repeat: matches 2648..2698 of consensus"
repeat_region   83963..84024
```

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                     /note="MIR repeat: matches 77..140 of consensus"
     repeat_region   84133..84258
                     /note="MIR repeat: matches 7..136 of consensus"
     repeat_region   84602..84697
                     /note="MIR repeat: matches 49..135 of consensus"
     repeat_region   85012..85350
                     /note="MLT1A2 repeat: matches 1..340 of consensus"
     repeat_region   85365..85456
                     /note="MIR repeat: matches 33..128 of consensus"
     repeat_region   85526..85776
                     /note="MIR repeat: matches 7..262 of consensus"
     repeat_region   85935..86011
                     /note="MER58A repeat: matches 42..121 of consensus"
     repeat_region   86101..86173
                     /note="MER58A repeat: matches 143..219 of consensus"
     repeat_region   87299..87643
                     /note="AluYb8 repeat: matches 1..310 of consensus"
     repeat_region   88003..88303
                     /note="AluSx repeat: matches 1..301 of consensus"
     repeat_region   92655..92799
                     /note="L1MC5 repeat: matches 7720..7866 of consensus"
     repeat_region   99017..99109
                     /note="L2 repeat: matches 2388..2488 of consensus"
     repeat_region   99392..99521
                     /note="L2 repeat: matches 2576..2710 of consensus"
     repeat_region   99694..99834
                     /note="L2 repeat: matches 2610..2750 of consensus"
     repeat_region   100051..100090
                     /note="20 copies 2 mer tt 80% conserved"
     repeat_region   100093..100400
                     /note="AluSx repeat: matches 3..312 of consensus"
     repeat_region   100764..100931
                     /note="MER3 repeat: matches 3..207 of consensus"
     repeat_region   101144..101446
                     /note="AluJo repeat: matches 1..292 of consensus"
     repeat_region   101798..101867
                     /note="L2 repeat: matches 2672..2744 of consensus"
     repeat_region   101952..102471
                     /note="MER1A repeat: matches 6..527 of consensus"
     repeat_region   102850..103006
                     /note="MIR repeat: matches 20..168 of consensus"
     repeat_region   103007..103052
                     /note="MLT1A1 repeat: matches 1..51 of consensus"
     repeat_region   103053..103356
                     /note="AluSp repeat: matches 1..303 of consensus"
     repeat_region   103357..103729
                     /note="MLT1A1 repeat: matches 51..365 of consensus"
     repeat_region   103730..103791
                     /note="MIR repeat: matches 168..231 of consensus"
     repeat_region   103886..103998
                     /note="MSTA repeat: matches 1..114 of consensus"
     repeat_region   103996..104323
                     /note="MSTA repeat: matches 1..388 of consensus"
     repeat_region   104372..104503
                     /note="AluY repeat: matches 166..297 of consensus"
     repeat_region   104594..104824
                     /note="MIR repeat: matches 10..259 of consensus"
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repeat_region   104874..104954
                /note="L2 repeat: matches 2624..2709 of consensus"
repeat_region   105235..105332
                /note="MIR repeat: matches 47..145 of consensus"
repeat_region   107333..107459
                /note="MLT1C repeat: matches 340..466 of consensus"
repeat_region   107460..107589
                /note="L1MA7 repeat: matches 6159..6288 of consensus"
repeat_region   107590..107718
                /note="MLT1C repeat: matches 211..340 of consensus"
repeat_region   107755..108017
                /note="AluJo repeat: matches 18..277 of consensus"
repeat_region   108019..108208
                /note="MLT1C repeat: matches 1..192 of consensus"
repeat_region   108326..108922
                /note="L2 repeat: matches 2080..2710 of consensus"
repeat_region   109592..109635
                /note="22 copies 2 mer gg 75% conserved"
repeat_region   110302..110511
                /note="L1MA10 repeat: matches 5970..6317 of consensus"
repeat_region   110589..111071
                /note="L1ME1 repeat: matches 5477..5952 of consensus"
repeat_region   111077..111135
                /note="L1MB4 repeat: matches 6124..6183 of consensus"
repeat_region   111153..111414
                /note="L1MB3 repeat: matches 5909..6182 of consensus"
repeat_region   111419..111617
                /note="L1ME1 repeat: matches 5283..5487 of consensus"
repeat_region   111622..112876
                /note="L1MB8 repeat: matches 4877..6173 of consensus"
repeat_region   112873..114198
                /note="L1M4 repeat: matches 3073..4391 of consensus"
repeat_region   114206..115694
                /note="L1PA2 repeat: matches 4656..6144 of consensus"
repeat_region   115723..116219
                /note="MLT2CA repeat: matches 1..489 of consensus"
repeat_region   116220..116291
                /note="3 copies 24 mer 81% conserved"
repeat_region   116283..116348
                /note="MLT2CA repeat: matches 444..508 of consensus"
repeat_region   116377..118409
                /note="L1MEc repeat: matches 1212..2985 of consensus"
repeat_region   118410..118683
                /note="AluY repeat: matches 1..298 of consensus"
repeat_region   118684..118923
                /note="L1MEc repeat: matches 983..1212 of consensus"
repeat_region   118955..119117
                /note="MER20 repeat: matches 51..218 of consensus"
repeat_region   119118..119284
                /note="L1MEc repeat: matches 802..988 of consensus"
repeat_region   119285..119682
                /note="MLT1A1 repeat: matches 1..365 of consensus"
repeat_region   119683..119810
                /note="L1MEc repeat: matches 681..802 of consensus"
repeat_region   119830..120493
                /note="L2 repeat: matches 1734..2603 of consensus"
repeat_region   120522..120894
```

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                /note="MLT1B repeat: matches 1..426 of consensus"
repeat_region   120923..121042
                /note="5 copies 24 mer 68% conserved"
repeat_region   121180..121227
                /note="2 copies 24 mer 95% conserved"
repeat_region   121184..121227
                /note="22 copies 2 mer gt 100% conserved"
repeat_region   122617..122772
                /note="MER5A repeat: matches 4..188 of consensus"
repeat_region   122787..122900
                /note="MER5B repeat: matches 91..173 of consensus"
repeat_region   122901..123193
                /note="AluJo repeat: matches 4..298 of consensus"
repeat_region   123194..123275
                /note="MER5B repeat: matches 1..91 of consensus"
repeat_region   123437..124087
                /note="L2 repeat: matches 1555..2750 of consensus"
repeat_region   124164..124586
                /note="L2 repeat: matches 57..485 of consensus"
repeat_region   125405..125535
                /note="Charlie4a repeat: matches 369..495 of consensus"
repeat_region   125536..125695
                /note="FRAM repeat: matches 2..161 of consensus"
repeat_region   125696..126026
                /note="Charlie4a repeat: matches 19..369 of consensus"
repeat_region   126098..126459
                /note="MLT1A1 repeat: matches 1..365 of consensus"
repeat_region   126630..126693
                /note="L2 repeat: matches 2641..2704 of consensus"
repeat_region   126784..127362
                /note="L2 repeat: matches 2176..2750 of consensus"
repeat_region   127366..127489
                /note="MIR repeat: matches 46..185 of consensus"
repeat_region   127679..127826
                /note="L2 repeat: matches 2552..2691 of consensus"
repeat_region   127827..128126
                /note="AluJo repeat: matches 4..302 of consensus"
repeat_region   128127..128405
                /note="L2 repeat: matches 2241..2552 of consensus"
repeat_region   128518..128663
                /note="L2 repeat: matches 1873..2021 of consensus"
repeat_region   128674..129015
                /note="MLT1A1 repeat: matches 1..358 of consensus"
repeat_region   129016..129205
                /note="L2 repeat: matches 1672..1868 of consensus"
repeat_region   129216..129297
                /note="L2 repeat: matches 2639..2710 of consensus"
repeat_region   129340..129554
                /note="MIR repeat: matches 53..260 of consensus"
repeat_region   129661..129924
                /note="MIR repeat: matches 2..261 of consensus"
repeat_region   131041..131232
                /note="AluSg/x repeat: matches 135..292 of consensus"
repeat_region   131267..131548
                /note="AluY repeat: matches 18..311 of consensus"
repeat_region   131743..132226
                /note="MLT1G repeat: matches 31..496 of consensus"
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repeat_region   132996..133294
                /note="AluSx repeat: matches 1..298 of consensus"
repeat_region   133642..133701
                /note="MIR repeat: matches 69..128 of consensus"
repeat_region   134184..134313
                /note="L1P3 repeat: matches 5682..5809 of consensus"
repeat_region   134373..134437
                /note="L1MA7 repeat: matches 6224..6288 of consensus"
repeat_region   134438..134744
                /note="MER2 repeat: matches 34..345 of consensus"
repeat_region   134748..134835
                /note="L1MA6 repeat: matches 6154..6241 of consensus"
repeat_region   134878..135187
                /note="AluSx repeat: matches 1..310 of consensus"
repeat_region   135493..135866
                /note="L1MD2 repeat: matches 5962..6340 of consensus"
repeat_region   135888..136007
                /note="L1ME1 repeat: matches 6026..6135 of consensus"
repeat_region   136270..136561
                /note="AluSx repeat: matches 21..312 of consensus"
repeat_region   136598..136661
                /note="MIR repeat: matches 69..132 of consensus"
repeat_region   136902..137090
                /note="MIR repeat: matches 12..206 of consensus"
repeat_region   137099..137227
                /note="MLT1D repeat: matches 1..130 of consensus"
repeat_region   137228..137524
                /note="AluSx repeat: matches 1..296 of consensus"
repeat_region   137525..137870
                /note="MLT1D repeat: matches 130..471 of consensus"
repeat_region   137893..138056
                /note="AluJo repeat: matches 123..301 of consensus"
repeat_region   138057..138345
                /note="AluSx repeat: matches 5..292 of consensus"
repeat_region   138346..138472
                /note="AluJo repeat: matches 1..123 of consensus"
repeat_region   138474..138539
                /note="MLT1D repeat: matches 440..505 of consensus"
repeat_region   138554..138675
                /note="L2 repeat: matches 2066..2187 of consensus"
repeat_region   138694..139033
                /note="L2 repeat: matches 2362..2692 of consensus"
repeat_region   138993..139044
                /note="MIR repeat: matches 210..261 of consensus"
repeat_region   139737..139807
                /note="MLT1C repeat: matches 393..464 of consensus"
repeat_region   139808..139873
                /note="L1PA8 repeat: matches 6097..6162 of consensus"
repeat_region   139874..140272
                /note="MLT1C repeat: matches 1..393 of consensus"
repeat_region   140332..140461
                /note="MER5A repeat: matches 26..189 of consensus"
repeat_region   140611..140903
                /note="LTR16C repeat: matches 61..387 of consensus"
repeat_region   141410..141594
                /note="MIR repeat: matches 25..210 of consensus"
repeat_region   142664..142699
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                     /note="18 copies 2 mer tc 100% conserved"
     repeat_region   142714..142995
                     /note="AluSc repeat: matches 1..278 of consensus"
     repeat_region   143097..143180
                     /note="L2 repeat: matches 2643..2749 of consensus"
     repeat_region   143339..143662
                     /note="MLT1A1 repeat: matches 6..365 of consensus"
     repeat_region   143783..143885
                     /note="L2 repeat: matches 2593..2702 of consensus"
     repeat_region   145806..145889
                     /note="MIR repeat: matches 37..130 of consensus"
     repeat_region   145957..146261
                     /note="AluSq repeat: matches 1..308 of consensus"
     repeat_region   146264..146376
                     /note="MIR repeat: matches 117..235 of consensus"
     repeat_region   147583..147657
                     /note="L2 repeat: matches 2422..2502 of consensus"
     repeat_region   147859..147978
                     /note="L2 repeat: matches 2616..2750 of consensus"
     repeat_region   148013..148084
                     /note="L2 repeat: matches 2636..2707 of consensus"
     repeat_region   148687..149141
                     /note="LTR16B repeat: matches 1..461 of consensus"
     repeat_region   150442..151102
                     /note="L2 repeat: matches 1490..2109 of consensus"
BASE COUNT
ORIGIN

468 100   0.0
AF195953    Homo sapiens membrane-bound aminopeptidase P (XNPEP2) gene,
          complete cds. 206618 bp, DNA, linear, PRI 26-MAR-2002
ACCESSION   AF195953
VERSION     AF195953.2  GI:19718557
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
          Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
          Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 206618)
  AUTHORS   Ryan,J.W., Jin,L., Horvath,I. and Sprinkle,T.J.C.
  TITLE     Human membrane-bound aminopeptidase P genomic DNA
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 206618)
  AUTHORS   Ryan,J.W., Jin,L., Horvath,I. and Sprinkle,T.J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (18-OCT-1999) Vascular Biology Center, Medical College of
          Georgia, 1120 15th Street, Augusta, GA 30912, USA
REFERENCE   3  (bases 1 to 206618)
  AUTHORS   Ryan,J.W., Jin,L., Horvath,I. and Sprinkle,T.J.C.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-MAR-2002) Vascular Biology Center, Medical College of
          Georgia, 1120 15th Street, Augusta, GA 30912, USA
  REMARK    Sequence update by submitter
COMMENT     On Mar 26, 2002 this sequence version replaced gi:11066156.
FEATURES             Location/Qualifiers
     source          1..206618
                     /organism="Homo sapiens"
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                     /mol_type="genomic DNA"
                     /db_xref="taxon:9606"
     gene              144189..176791
                     /gene="XNPEP2"
     mRNA              join(144189..144502,147348..147421,149203..149313,
                     150445..150508,151478..151582,151837..151923,
                     152849..152995,155710..155811,156987..157068,
                     157392..157587,158401..158490,159714..159823,
                     160536..160613,161726..161797,164422..164482,
                     165754..165823,166414..166518,167248..167307,
                     167936..168012,172845..172934,173533..176791)
                     /gene="XNPEP2"
                     /product="membrane-bound aminopeptidase P"
     5'UTR             144189..144453
                     /gene="XNPEP2"
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                     152849..152995,155710..155811,156987..157068,
                     157392..157587,158401..158490,159714..159823,
                     160536..160613,161726..161797,164422..164482,
                     165754..165823,166414..166518,167248..167307,
                     167936..168012,172845..172934,173533..173727)
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                     /codon_start=1
                     /product="membrane-bound aminopeptidase P"
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                     /db_xref="GI:11066157"
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                     /gene="XNPEP2"
                     /note="putative proton shuttle; unclassified site"
     misc_feature      161778..161780
                     /gene="XNPEP2"
                     /note="divalent metal ligand; metal-binding site"
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                     /gene="XNPEP2"
                     /note="divalent metal ligand; metal-binding site"
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                     /note="divalent metal ligand; metal-binding site"
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                     /gene="XNPEP2"
                     /note="divalent metal ligand; metal-binding site"
     misc_feature      167977..167979
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                     /note="divalent metal ligand; metal-binding site"
     3'UTR             175728..176791
                     /gene="XNPEP2"
BASE COUNT
ORIGIN

467  99   0.0
P_AAH14799  Human cDNA sequence SEQ ID NO:12589. 243 bp, cDNA, PAT 26-JUN-2001
ACCESSION   P_AAH14799
KEYWORDS    GENESEQ; Human; primer; detection; diagnosis; antisense therapy;
          gene therapy;  patent; patentdb (v200414, 01-JUL-2004).
SOURCE      Homo sapiens.
  ORGANISM  Homo sapiens.
```

REFERENCE 1 (bases 1 to 2243)
AUTHORS Ota,T., Isogai,T., Nishikawa,T., Hayashi,K., Saito,K., Yamamoto,J. Ishii,S., Sugiyama,T., Wakamatsu,A., Nagai,K., Otsuki,T.
TITLE Primer sets for synthesizing polynucleotides, particularly the 5602 full- length cDNAs defined in the specification, and for the detection and/or diagnosis of the abnormality of the proteins encoded by the full-length cDNAs.
JOURNAL Patent: EP1074617-A2; Filing Date: 28-JUL-2000; 2000EP-00116126; Publication Date: 07-FEB-2001; Priority: 29-JUL-1999; 99JP-00248036. 27-AUG-1999; 99JP-00300253. 11-JAN-2000; 2000JP-00118776. 02-MAY-2000; 2000JP-00183767. 09-JUN-2000; 2000JP-00241899; Assignee: (HELI-) HELIX RES INST; Cross Reference: WPI; 2001-318749/34; Patent Format: Claim 8; SEQ ID NO 12589; 2537pp + Sequence Listing; English.
COMMENT The present invention describes primer sets for synthesising 5602 full- length cDNAs defined in the specification. Where a primer set comprises: (a) an oligo-dT primer and an oligonucleotide complementary to the complementary strand of a polynucleotide which comprises one of the 5602 nucleotide sequences defined in the specification, where the oligonucleotide comprises at least 15 nucleotides; or (b) a combination of an oligonucleotide comprising a sequence complementary to the complementary strand of a polynucleotide which comprises a 5'-end sequence and an oligonucleotide comprising a sequence complementary to a polynucleotide which comprises a 3'-end sequence, where the oligonucleotide comprises at least 15 nucleotides and the combination of the 5'-end sequence/3'-end sequence is selected from those defined in the specification. The primer sets can be used in antisense therapy and in gene therapy. The primers are useful for synthesising polynucleotides, particularly full-length cDNAs. The primers are also useful for the detection and/or diagnosis of the abnormality of the proteins encoded by the full-length cDNAs. The primers allow obtaining of the full-length cDNAs easily without any specialised methods. AAH03166 to AAH13628 and AAH13633 to AAH18742 represent human cDNA sequences; AAB92446 to AAB95893 represent human amino acid sequences; and AAH13629 to AAH13632 represent oligonucleotides, all of which are used in the exemplification of the present invention
FEATURES Location/Qualifiers
BASE COUNT 467 a 630 c 637 g 509 t
ORIGIN

467 99 0.0
AK001855 Homo sapiens cDNA FLJ10993 fis, clone PLACE1002140. 2243 bp, mRNA, linear, PRI 30-JAN-2004
ACCESSION AK001855
VERSION AK001855.1 GI:7023382
KEYWORDS oligo capping; fis (full insert sequence).
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1
AUTHORS Ota,T., Suzuki,Y., Nishikawa,T., Otsuki,T., Sugiyama,T., Irie,R., Wakamatsu,A., Hayashi,K., Sato,H., Nagai,K., Kimura,K., Makita,H., Sekine,M., Obayashi,M., Nishi,T., Shibahara,T., Tanaka,T.,

Ishii,S., Yamamoto,J., Saito,K., Kawai,Y., Isono,Y., Nakamura,Y., Nagahari,K., Murakami,K., Yasuda,T., Iwayanagi,T., Wagatsuma,M., Shiratori,A., Sudo,H., Hosoiri,T., Kaku,Y., Kodaira,H., Kondo,H., Sugawara,M., Takahashi,M., Kanda,K., Yokoi,T., Furuya,T., Kikkawa,E., Omura,Y., Abe,K., Kamihara,K., Katsuta,N., Sato,K., Tanikawa,M., Yamazaki,M., Ninomiya,K., Ishibashi,T., Yamashita,H., Murakawa,K., Fujimori,K., Tanai,H., Kimata,M., Watanabe,M., Hiraoka,S., Chiba,Y., Ishida,S., Ono,Y., Takiguchi,S., Watanabe,S., Yosida,M., Hotuta,T., Kusano,J., Kanehori,K., Takahashi-Fujii,A., Hara,H., Tanase,T., Nomura,Y., Togiya,S., Komai,F., Hara,R., Takeuchi,K., Arita,M., Imose,N., Musashino,K., Yuuki,H., Oshima,A., Sasaki,N., Aotsuka,S., Yoshikawa,Y., Matsunawa,H., Ichihara,T., Shiohata,N., Sano,S., Moriya,S., Momiyama,H., Satoh,N., Takami,S., Terashima,Y., Suzuki,O., Nakagawa,S., Senoh,A., Mizoguchi,H., Goto,Y., Shimizu,F., Wakebe,H., Hishigaki,H., Watanabe,T., Sugiyama,A., Takemoto,M., Kawakami,B., Yamazaki,M., Watanabe,K., Kumagai,A., Itakura,S., Fukuzumi,Y., Fujimori,Y., Komiyama,M., Tashiro,H., Tanigami,A., Fujiwara,T., Ono,T., Yamada,K., Fujii,Y., Ozaki,K., Hirao,M., Ohmori,Y., Kawabata,A., Hikiji,T., Kobatake,N., Inagaki,H., Ikema,Y., Okamoto,S., Okitani,R., Kawakami,T., Noguchi,S., Itoh,T., Shigeta,K., Senba,T., Matsumura,K., Nakajima,Y., Mizuno,T., Morinaga,M., Sasaki,M., Togashi,T., Oyama,M., Hata,H., Watanabe,M., Komatsu,T., Mizushima-Sugano,J., Satoh,T., Shirai,Y., Takahashi,Y., Nakagawa,K., Okumura,K., Nagase,T., Nomura,N., Kikuchi,H., Masuho,Y., Yamashita,R., Nakai,K., Yada,T., Nakamura,Y., Ohara,O., Isogai,T. and Sugano,S.

TITLE Complete sequencing and characterization of 21,243 full-length human cDNAs

JOURNAL Nat. Genet. 36 (1), 40-45 (2004)

PUBMED 14702039

REFERENCE 2

AUTHORS Isogai,T., Ota,T., Hayashi,K., Sugiyama,T., Otsuki,T., Suzuki,Y., Nishikawa,T., Nagai,K., Sugano,S., Takahashi-Fujii,A., Hara,H., Tanase,T., Nomura,Y., Togiya,S., Komai,F., Hara,R., Takeuchi,K., Arita,M., Nabekura,T., Ishii,S., Kawai,Y., Saito,K., Yamamoto,J., Wakamatsu,A., Nakamura,Y., Nagahari,K., Masuho,Y. and Oshima,A.

TITLE NEDO human cDNA sequencing project

JOURNAL Unpublished

REFERENCE 3 (bases 1 to 2243)

AUTHORS Isogai,T. and Otsuki,T.

TITLE Direct Submission

JOURNAL Submitted (16-FEB-2000) Takao Isogai, Helix Research Institute, Genomics Laboratory; 1532-3 Yana, Kisarazu, Chiba 292-0812, Japan (E-mail:genomics@hri.co.jp, Tel:81-438-52-3975, Fax:81-438-52-3986)

COMMENT NEDO human cDNA sequencing project supported by Ministry of International Trade and Industry of Japan; cDNA full insert sequencing: Research Association for Biotechnology; cDNA library construction, 5'- & 3'-end one pass sequencing and clone selection: Helix Research Institute (supported by Japan Key Technology Center etc.) and Department of Virology, Institute of Medical Science, University of Tokyo.

FEATURES Location/Qualifiers

source 1..2243

/organism="Homo sapiens"

/mol_type="mRNA"

/db_xref="taxon:9606"

/clone="PLACE1002140"

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                     /tissue_type="placenta"
                     /clone_lib="PLACE1"
                     /note="cloning vector: pME18SFL3"
     CDS             6..374
                     /note="unnamed protein product"
                     /codon_start=1
                     /protein_id="BAA91944.1"
                     /db_xref="GI:7023383"
BASE COUNT
ORIGIN

449  98   0.0
AX332625    Sequence 3134 from Patent WO0194629. 458 bp,
          DNA, linear, PAT 09-JAN-2002
ACCESSION   AX332625
VERSION     AX332625.1  GI:18123259
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
          Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
          Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Young,P.E., Augustus,M., Carter,K.C., Ebner,R., Endress,G.,
          Horrigan,S., Soppet,D.R. and Weaver,Z.
  TITLE     Cancer gene determination and therapeutic screening using signature
          gene sets
  JOURNAL   Patent: WO 0194629-A 3134 13-DEC-2001;
          Avalon Pharmaceuticals (US)
FEATURES             Location/Qualifiers
     source          1..458
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                     /db_xref="taxon:9606"
BASE COUNT
ORIGIN

449  98   0.0
AX332852    Sequence 3361 from Patent WO0194629. 458 bp,
          DNA; linear, PAT 09-JAN-2002
ACCESSION   AX332852
VERSION     AX332852.1  GI:18123486
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
          Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
          Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1
  AUTHORS   Young,P.E., Augustus,M., Carter,K.C., Ebner,R., Endress,G.,
          Horrigan,S., Soppet,D.R. and Weaver,Z.
  TITLE     Cancer gene determination and therapeutic screening using signature
          gene sets
  JOURNAL   Patent: WO 0194629-A 3361 13-DEC-2001;
          Avalon Pharmaceuticals (US)
FEATURES             Location/Qualifiers
     source          1..458
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                     /mol_type="unassigned DNA"
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/db_xref="taxon:9606"

BASE COUNT
ORIGIN

Dayhoff Protein Database (Rel 78, Mar 2004)

P_AAB87540 Human PRO831 - Homo sapiens.
Length: 73 aa
Accession: P_AAB87540;
Species: Homo sapiens.
Keywords: Human; PRO protein; mapping; patent; GENESEQ patentdb.
Patent number: WO200116318-A2.
Publication date: 08-MAR-2001.
Filing date: 24-AUG-2000; 2000WO-US023328.
Priority: 01-SEP-1999; 99WO-US020111. 15-SEP-1999; 99WO-US021090.
07-DEC-1999; 99US-0169495P. 09-DEC-1999; 99US-0170262P.
11-JAN-2000; 2000US-0175481P. 18-FEB-2000; 2000WO-US004341.
18-FEB-2000; 2000WO-US004342. 22-FEB-2000; 2000WO-US004414.
01-MAR-2000; 2000WO-US005601. 03-MAR-2000; 2000US-0187202P.
21-MAR-2000; 2000US-0191007P. 30-MAR-2000; 2000WO-US008439.
25-APR-2000; 2000US-0199397P. 22-MAY-2000; 2000WO-US014042.
05-JUN-2000; 2000US-0209832P.
Assignee: (GETH) GENENTECH INC.
Inventors: Eaton DL, Filvaroff E, Gerritsen ME, Goddard A, Godowski PJ;
Grimaldi CJ, Gurney AL, Watanabe CK, Wood WI;
Cross reference: WPI; 2001-183260/18. N-PSDB; AAF92072.
Title: Eighty four nucleic acids encoding PRO polypeptides, useful in molecular biology, including use as hybridization probes, and in chromosome and gene mapping.
Patent format: Claim 12; Fig 30; 278pp; English.
Comment: The present sequence is a human PRO polypeptide (secreted and transmembrane). The PRO protein, and PRO agonists, PRO antagonists or anti-PRO antibodies are useful for preparation of a medicament useful in the treatment of a condition which is responsive to the PRO protein, agonists, antagonists or anti-PRO antibodies. The PRO protein may also be employed as molecular weight markers for protein electrophoresis. The PRO coding sequence has applications in molecular biology, including use as hybridisation probes, and in chromosome and gene mapping
Database: GENESEQ patent database (v200414, 01-JUL-2004).

P_AAY99346 Human PRO831 (UNQ471) amino acid sequence SEQ ID NO:22 - Homo sapiens.
Length: 73 aa
Accession: P_AAY99346;
Species: Homo sapiens.
Keywords: Human; PRO polypeptide; membrane bound protein; receptor; diagnosis; transmembrane; secretion; immunoadhesion; pharmaceutical; screening; patent; GENESEQ patentdb.
Patent number: WO200012708-A2.
Publication date: 09-MAR-2000.
Filing date: 01-SEP-1999; 99WO-US020111.
Priority: 01-SEP-1998; 98US-0098716P. 01-SEP-1998; 98US-0098749P.
01-SEP-1998; 98US-0098750P.18-NOV-1998; 98US-0108858P. 18-NOV-1998; 98US-0108904P. plus 119 more dates.
Assignee: (GETH) GENENTECH INC.
Inventors: Baker K, Goddard A, Gurney AL, Smith V, Watanabe CK, Wood WI;
Cross reference: WPI; 2000-237871/20. N-PSDB; AAA37028.
Title: New mammalian DNA sequences encoding transmembrane, receptor or secreted PRO polypeptides, useful for screening of potential peptide or small molecule inhibitors of the relevant

receptor/ligand interactions.
Patent format: Claim 12; Fig 14; 773pp; English.
Comment: AAA37022 to AAA37144 encode the new isolated human transmembrane, receptor or secreted PRO polypeptides given in AAY99340 to AAY99462. The transmembrane and receptor PRO proteins can be used for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interactions. The polypeptides and nucleotide sequences encoding then have various industrial applications, including uses as pharmaceutical and diagnostic agents. AAA37145 to AAA37330 represent PCR primers and hybridisation probes used in the isolation of the PRO polypeptides from the present invention
Database: GENESEQ patent database (v200414, 01-JUL-2004).